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SERUMS, VACCINES AND TOXINES

IN TREATMENT AND DIAGNOSIS

BY

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ILLUSTRATED

SECOND EDITION THOROUGHLY REVISED

NEW YORK

FUNK AND WAGNALLS COMPANY

1910

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PREFACE TO THE SECOND EDITION

JUST five years have elapsed since the first edition of this little book was published, and meanwhile the progress of investigation in the field of immunity has been continuous. A complete revision of the text was therefore necessitated, and considerable additions have been made. Perhaps the most notable advance has been in our experience of treatment by so-called vaccines—a somewhat unfortunate term in that the dead cultures of micro-organisms which are used have nothing to do with cows, nor is the procedure directly analogous to vaccination, which is performed with a living virus. The term, however, seems too firmly established to be altered now. Progress in this field has been very largely due to the stimulating work of Sir Almroth Wright and his assistants, to whom is also due the invention of a method of control of such treatment by observations of the opsonic or phagocytic index, the value of which has still to be determined. Closely connected with these procedures is the increased attention recently paid to tuberculin as a remedy for tubercular disease of all kinds, a more suitable scale of doses having been devised and the dangers inherent in the old scheme of treatment thus practically banished.

The application of vaccines to acute as opposed to chronic diseases is still in its infancy, but sufficient evidence already exists to awaken hopes of a useful extension of the procedure to this field. It is unfortunate that so far it has not been found possible to apply any form of vaccination

or serum-therapeutics to those protozoal diseases which have in late years come prominently into notice. In the direction of diagnosis, however, the method known as "fixation of complement" seems likely to afford valuable assistance in the recognition of syphilitic infection.

For the present, it must be confessed that antibacterial serums have proved disappointing; and while diphtherial antitoxine has been placed beyond criticism as an indispensable remedy, the few other antitoxic serums occupy but a subordinate position in medical practice.

Owing to the amount of new material added to the present edition, it has been found necessary to omit a considerable portion of the introductory matter. It has thus been possible to retain almost the original size of the volume.

W. C. B.
J. E.

November, 1909.

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SERUMS, VACCINES, AND TOXINES

IN

TREATMENT AND DIAGNOSIS

CHAPTER I

IMMUNITY AND RESISTANCE TO DISEASE

Acquired resistance.—The problem of the nature of disease and of the manner in which living bodies resist and recover from its attacks, has exercised the minds of mankind since very early times, and the fundamental truth that in the case of certain diseases one attack protects against subsequent infection was long ago discovered. The first attempt to utilise this principle for prophylactic purposes was made in the East in the form of inoculation against small-pox, a mild infection being produced by inoculation with matter derived from a pustule, in order to ward off danger of subsequent infection. Shortly after the introduction of this procedure into the West of Europe, Jenner's great discovery that vaccination with cow-pox was equally efficacious as a protection and practically free from risk, laid the foundation of our knowledge of therapeutic inoculation. It was only, however, after the discovery of the minute living agents which cause infective diseases had been rendered possible by improvements in optical science that further advance in this field was made. When bacteria were recognised as the cause of most infective diseases, and their mode of action by the secretion of poisons or toxines was ascertained, research into the mode of resistance to these invaders was stimulated; and from the time of

Koch's invention of accurate methods for the separation and identification of bacteria, followed as it was at no distant date by Behring and Kitasato's work on antitoxines and by Pfeiffer's experiments in bacteriolysis, knowledge of the various factors involved in immunity has steadily advanced.

Phagocytosis.—The first important theory of resistance to disease was that of Metchnikoff (1865–1884), who studied the behaviour of the white blood-corpuscles (leucocytes) in many of the lower animals, and attributed the destruction of bacteria in the body to the activity of these cells. This was the well-known theory of "*phagocytosis*" (φαγεῖν, to eat; κύτος, a cell). According to Metchnikoff, the leucocytes attack and devour any invading organisms which they may meet, and thus rid the body of these parasites, just as they may be seen to take into their substance particles of any foreign matter which comes in their way. When they have swallowed and thus destroyed all the bacteria which have gained a footing in the body, the disease necessarily comes to an end.

The careful and minute study carried out by the French observer cannot be too much admired, and there can be no doubt that it contains a large proportion of truth. Thus, the assemblage of leucocytes which takes place at any focus of irritation is almost certainly protective in character; and it has been shown by Kanthack and others that the granules contained in the protoplasm of the leucocytes consist of substances which tend to combat the bacteria and to stop their growth. But in man, at all events, this phagocytic action is not the sole factor in the struggle with the invading germs.

Further experiments showed that the serum of the blood, even when all formed elements, such as the corpuscles, had been removed, still exerted in many instances an inhibitory action on the growth of micro-organisms (Nuttall, Büchner). There must, therefore, be present in the plasma some substance of a protective nature, and to these

hypothetical substances Büchner gave the name "alexines." It is by means of such alexines, as will shortly be shown, that destruction of bacteria is in many cases brought about; and it is by other chemical substances circulating in the blood that the poisonous products of the organisms are neutralised.

As a result of these discoveries attention was directed for a time mainly to the chemical contents of the serum and other fluids, and the importance of the process of phagocytosis was seriously called in question. A whole series of peculiar properties possessed by the serum of immunised animals was brought to light, and was used to support the "humoral theory" of immunity. Here, however, as in many other instances in which opposing theories have been hotly upheld and attacked, time has shown that each side in the controversy had grasped a portion of the truth, and declined in the heat of conflict to recognise the other portion which was defended by their antagonists. The upholders of the phagocytic hypothesis satisfactorily proved that in many important affections the serum alone was ineffective in destroying bacteria and that the action of leucocytes was essential for the process of defence, and maintained further that the very chemical bodies which were held by their opponents to constitute the basis of immunity, were themselves secreted by the leucocytes of the blood. The discovery of a special group of substances ("opsonins," p. 34) existing in the serum, by which the process of phagocytosis is induced or at least facilitated, has to some extent reconciled the positions of the opposing schools. But, so far as is at present known, it would appear that both phagocytosis and bacteriolysis (p. 6) take part in the destruction of bacteria within the body, now one, now the other predominating, according to the nature of the infective agent.

Antitoxines.—In the year 1890 Behring and Kitasato published the result of their important researches on the poison of *tetanus* and on the possibility of rendering animals immune to it. These observers proved that it was possible

by injecting animals first with infinitesimal quantities, later with increasing doses, of the toxines of tetanus to render them immune to the disease. The animals thus treated were able to support with impunity doses of the tetanus-poison many times as great as would suffice to kill an ordinary non-immunised animal of the same species. If the serum of an immunised animal were mixed with an equivalent amount of the poison and injected into a non-immune animal, no ill effects were produced; while the injection of the immune serum itself into a non-immunised animal rendered the latter also resistant to the toxine. If a dose of immune serum was administered within a short period of time to an animal previously inoculated with the tetanus-bacillus, the disease did not develop.

The same observers, and also Wernicke, shortly afterwards showed that similar possibilities existed with regard to the bacillus of *diphtheria*—that by treating animals with the toxines of this organism a serum could be obtained which was capable of neutralising the poison, and which also possessed a curative effect on the disease. To the unknown substance in the serum which had the property of neutralising the toxine they gave the name of "antitoxine." The antitoxic bodies formed in the two cases were not the same; the tetanus-antitoxine did not act as an antidote to the poison of diphtheria nor *vice versâ*. Each serum was "specific," neutralising only the poison of the corresponding disease; and this peculiarity has been found to exist in all subsequently prepared antitoxines.

In the light of these discoveries as to the reaction of living animals to bacterial toxines, attention was turned to the effects produced by other organic poisons, and it was found that it was possible to immunise animals to the vegetable poisons, abrin (from jequirity), ricin (castor oil), and crotin (croton oil), which are probably of complex proteid nature, and resemble ferments in their action. In the case of each of these substances it was possible to obtain a specific antitoxic serum, protecting only against its

appropriate toxine. Similarly, in the case of snake-venom an antitoxic serum was prepared, of which use has been made therapeutically with some degree of success.

Chemical nature of antitoxines.—Examination of the blood of horses used for the preparation of diphtherial antitoxine shows that the globulin-content of the serum is increased.[1] Further, if the antitoxic serum obtained from them is fractionally precipitated with ammonium sulphate, it is found that the antitoxine is precipitated with the pseudo-globulin[2]—that portion which is thrown down by semi-saturation with the salt. In animals other than horses (*e.g.* goats) the antitoxine may be thrown down with the euglobulin precipitate. Hence it has been inferred that the antitoxine is a globulin. This cannot be regarded as definitely proved, since substances are often carried down with precipitates from which they are chemically distinct (*e.g.* ferments). Proescher[3] believes that antitoxines are non-albuminous.

Antibacterial serum.—It may here be pointed out that in order to prepare an antitoxic serum it is necessary to obtain the toxine of the bacterium in question for the purpose of injection into animals. In the case of diphtheria and tetanus this was easily done. In the case of many organisms, however, difficulties arise, since their poison is not secreted into culture-media, but remains in the bodies of the bacteria themselves. If the actual germs are injected into animals, beginning with minute doses of attenuated cultures and gradually increasing until large quantities of virulent bacteria can be tolerated, in most cases a serum is produced which is not antitoxic in the sense of neutralising the poisons of the micro-organism, but which destroys the bacteria themselves when they are submitted to its action. Such a serum is said to be

[1] This is denied by Ledingham. *Journ. of Hygiene*, 1907, vii. 65, 92.

[2] Pick, *Hofmeister's Beitr.*, 1901, p. 1384.

[3] *Münch. med. Woch.*, 1902, p. 1176.

"antibacterial" or "bactericidal," instead of antitoxic. Thus, if an animal is injected with cholera-vibrios until it is very resistant to these germs, and then a little of its blood-serum is added to a culture of these organisms, the latter are found to undergo degeneration, and finally to be completely dis-

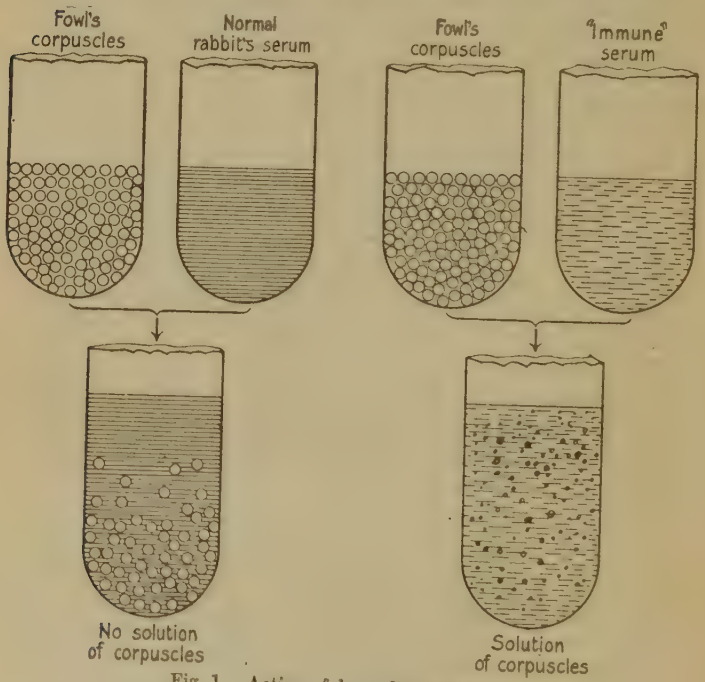

Fig. 1.—Action of hæmolytic serum.

integrated; but no quantity of this serum will neutralise a lethal dose of the poison of the cholera-germ. On the other hand, an antitoxic serum has no effect in preventing the growth of the appropriate organism; for instance, diphtherial antitoxine is a very favourable culture-medium for the Klebs-Loeffler bacillus. The process of destruction of bacteria by an antibacterial serum is called "bacteriolysis," and the property resides not only in the blood-serum, but

also in other vital fluids, such as the peritoneal exudate. It is evident that some special substance is produced in the bodies of the immunised animals which acts as a solvent of the bacterial protoplasm.

Hæmolysis.—Further research showed that it is not only bacteria which, by injection into living animals, give

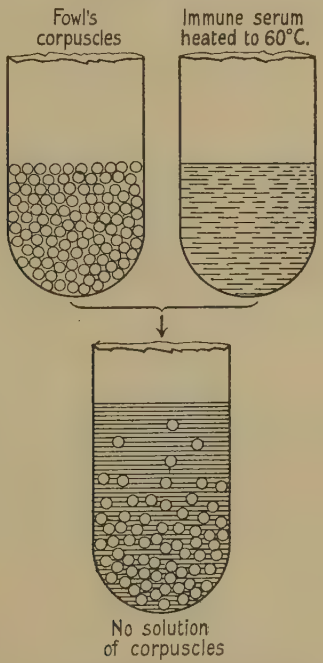

Fig. 2.—Effect of heat on hæmolytic serum (destruction of complement).

rise to the production of substances destructive to themselves. In 1891 Bordet, to whom much of our knowledge of the phenomena of bacteriolysis is due, discovered that, if the blood of one species of animal were injected into an individual of another kind, the serum of the latter developed the property of dissolving the corpuscles of animals of the former species. Thus, if the blood of a fowl

is injected into a rabbit, the serum of the rabbit gains the power of dissolving the corpuscles of fowl's blood, when added to it in a test-tube. This phenomenon is called "hæmolysis," and the hæmolytic power is exactly analogous to the bacteriolytic property in the cases previously described.

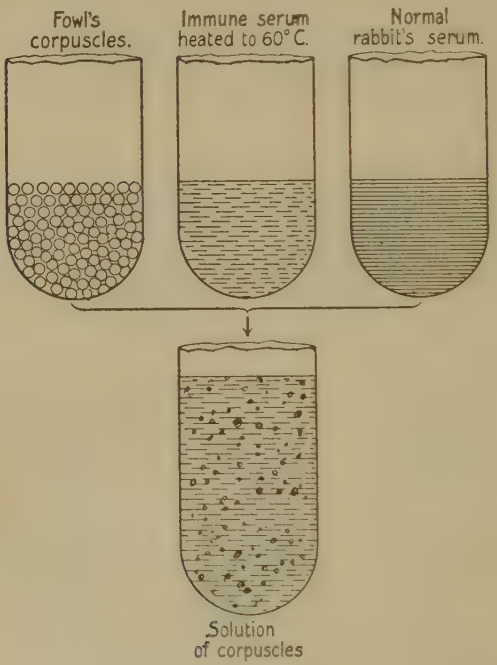

Fig. 3.—Addition of complement from normal serum to heated immune serum.

Copula and complement.—Now, if the hæmolytic serum of the rabbit in this experiment is heated, it is found to have lost its solvent power; but if a little serum from a normal rabbit is added to the heated serum, the property of dissolving the fowl's corpuscles returns to it.

The same occurs in the case of bacteriolytic serum. Thus Pfeiffer showed that in the peritoneal cavity of an

immunised guinea-pig cholera-vibrios undergo a process of destruction, and that the same occurs in a test-tube containing serum derived from such an animal. If this serum is heated, it loses its potency. If, however, a little of this heated serum is injected along with cholera-germs into the peritoneal cavity of a non-immune guinea-pig, solution takes place, the peritoneal fluid of the normal guinea-pig supplying the substance which was destroyed by the heat.

The accompanying diagrams (Figs. 1, 2, 3) will make these statements more clear.

From this it is apparent that in the process of hæmolysis the interaction of two bodies is necessary to bring about the result. One of these—the complement or alexine (*a*)—is present in normal serum, whereas the second—the copula or immune body (*b*)—is only developed in immunised animals. The former (*a*) is destroyed by heat, whereas the latter (*b*) is more stable, and is resistant to it. The complement is probably of ferment-like nature, and the process of destruction has been supposed to be one of hydrolysis (Turro).[1]

The multiplicity of names applied to these two bodies by different writers may possibly lead to some confusion. We may tabulate them thus :—

(*a*) **Complement** (always present), called also—	(*b*) **Copula** (developed in immunised animals), called also—
Alexine.	Intermediary body.
Addiment.	Immune body.
Cytase.	Amboceptor.
	Fixative.
	Mediator.
	Desmon.
	Préparateur.
	Substance sensibilitrice.
	Sensitising substance.

Perhaps the words *complement* and *copula* are as convenient as any to denote these substances respectively.

[1] *Berlin. klin. Woch.*, Sept. 7, 1903.

The foregoing experiments do not succeed if the serum and corpuscles be kept at a low temperature (0 C.). If, however, a mixture of hæmolytic serum and corpuscles is made and kept at this degree of temperature, and then the corpuscles are separated from the serum and washed clean by saline solution, it is found that they are now destroyed by the addition of a normal (non-hæmolytic) serum. This shows that the copula or intermediary body has become fixed to the corpuscles in some way, so that these are now "sensitive" to the action of the complement contained in normal serum. For this reason the copula has been called by French writers the "sensitiser" or "preparator" (*substance sensibilitrice : préparateur*). Use is made of corpuscles—or of bacteria, for the same occurs in their case also—thus sensitised, for the purpose of experiments, to which further allusion will be made on a subsequent page (*see* p. 29).

The serum of some animals is found to be actively destructive of that of other species, without any preliminary treatment by injection of blood. Thus the serum of the eel produces rapid hæmolysis if injected into mammalian animals, and is thus highly poisonous in its action. It is probable that in this case the intermediary body or copula necessary for the action upon blood-corpuscles of a ferment already existing in the blood, is supplied by the serum of the eel. In other cases minor degrees of the same toxicity may be observed, the serum of many animals exerting, without preliminary treatment, a hæmolytic action on the corpuscles of other species.

In some diseases hæmolytic substances may develop in human blood, capable of acting on the blood of another human individual (isolysins). The possibility of the existence in diseases characterised by extreme bloodlessness, such as pernicious and splenic anæmia, of substances of the nature of copulas which unite with the complement present and thus lead to the destruction of the patient's own blood-corpuscles, opens up an interesting field of speculation;

but there is as yet little definite evidence of the existence of such substances (autolysins).[1]

Cytolysis.—It is found that similar "antibodies" are produced by the injection, not only of bacteria and blood-corpuscles, but of many other kinds of cells, as, for instance, spermatozoa, nerve-cells, leucocytes, liver-cells, gastric epithelium,[2] &c. Serum from an animal thus injected with spermatozoa derived from another species is found to contain a substance (*cytolysin*) capable of destroying the spermatozoa existing within the living body of an individual of the latter species. The question of the possibility of preparing a serum which should be capable of destroying the cells of a tumour, *e.g.* a cancer, without affecting the normal epithelium, is of considerable interest in relation to the treatment of such disease (*see* p. 347).

Precipitation.—Very closely allied to this formation of cytolysins, or substances which are capable of dissolving cells, is the appearance of materials which act in a peculiar way on the soluble albuminous substances contained in serum itself. These are called *precipitins*, and are formed when the serum of one kind of animal is injected into the body of another species.[3] Thus, if the serum of, say, a horse is injected into a goat, the serum of the latter acquires the property of forming a precipitate with normal horse's serum. It appears that the precipitate is formed at the expense of the "immune" serum (*i.e.* that of the animal

[1] Eason finds that *paroxysmal hæmoglobinuria* is due to the action of a hæmolytic copula and complement, which are present in the blood of patients suffering from the disease and which unite with the corpuscles under the influence of cold (*Scot. Med. and Surg. Journ.*, May, 1906).

[2] Bolton, *Trans. Path. Soc.*, lvii., 1906, p. 297.

[3] The serum of animals treated by injection of the blood of another species possesses, in addition to its destructive action, a power of agglutinating the blood-corpuscles of the latter, *i.e.* causing them to adhere together. This property is not lost on heating the serum to 55° C. H. Marx and Ehrnrooth find that human corpuscles are agglutinated by the serum of any other animal, and suggest the use of this property as a medico-legal test (*Münch. med. Woch.*, Feb. 16, 1904, p. 293).

treated by injections of blood), not of the normal serum or albuminous fluid; and it was suggested by Uhlenhuth that use might be made of this fact to constitute a test for different kinds of blood. The possession of such a test for human blood would be of considerable medico-legal value. But unfortunately this particular test is not so absolutely specific as might be wished, as the serum of the injected animal is found to give a precipitate not only with the serum of the exact species of animal used to inoculate it, but also with the blood of allied kinds (*e.g.* apes and man). Further it must be borne in mind that the test is one for distinguishing between albuminous substances derived from different species, and not for blood alone.

Some authorities [1] deny that even this limited degree of specificity exists, finding that a precipitating substance, formed by injecting human blood into an animal—which therefore should act solely on human or anthropoid blood—will react also with that of oxen, horses, sheep, pigs, &c. The most pronounced action is, however, on human blood, and according to these authors error may be avoided by diluting the serum. Thus a precipitating serum diluted to 1 in 1000 will react only with the blood of the animal with which it was prepared. It is possible, therefore, that with this modification the test may still prove to possess a field of usefulness. The age of the blood used—stains on linen, &c.—does not affect the reaction.

Wassermann-Uhlenhuth test for blood.—Tchistowitsch [2] injected rabbits with the *serum* of horses and found that the rabbits' serum as a result of the injections acquired the power of precipitating part of the albumin of the horse-serum when mixed with it. Other observers amplified these results, and in consequence Wassermann proposed to use serum from animals previously injected with human serum to distinguish human from other blood. Uhlenhuth [3]

[1] Linessier and Lemoine, *Gaz. des Hôp.*, March 27, 1902.
[2] *Ann. Pasteur Inst.*, xiii., 1899, p. 406.
[3] *Berlin. klin. Woch.*, 1901, p. 187.

tested nineteen kinds of blood, and found that human blood alone gave the reaction. Stern[1] showed that monkey's blood gave a reaction similar to that produced by human blood. In order to prepare the antiserum a rabbit is first injected with sterile freshly defibrinated blood, or preferably with sterile serum, at intervals of four or five days for a period of two or three weeks. The animal is bled from a vein, and when the clot has separated the serum is pipetted off and stored in a cool place.

In performing the test the suspected blood is mixed with a small quantity of normal saline solution and filtered : the filtrate is divided between two test-tubes, to one of which is added twice the volume of antiserum. In another tube is placed some blood from some other mammal, together with antiserum, and in a fourth tube antiserum mixed with normal saline solution. All four tubes are incubated at 37° C. for one hour, then allowed to stand at room-temperature for four hours. If the suspected blood was of human origin the first tube alone will show evidence of precipitation, the remaining three control tubes being perfectly clear. The test is active even in extreme dilution—Stern quotes a positive reaction with blood diluted 50,000 times.

Agglutination.—When the serum of animals which have suffered from a disease or been inoculated with a micro-organism is added to a culture of the specific bacteria of the disease, it causes them to stick together in clumps or masses, instead of floating separately in the culture-fluid (Fig. 4). The best-known instance of this is the reaction produced by the serum of a patient suffering, or who has recovered, from enteric fever, when it is added to a culture of the *Bacillus typhosus*. It is found that if we take a young and vigorous culture of typhoid bacilli in broth, and add to it a small quantity of the serum of a patient with enteric fever, the bacilli almost immediately cease their normal active movement, and soon become collected together into clumps. The hypothetical bodies on which this reaction

[1] *Deut. med. Woch.*, 1901, p. 135.

depends are called "agglutinins." The discovery of this phenomenon is due to Gruber and Durham, and experiments were made with regard to its clinical possibilities by Grünbaum; but Widal first published his results, showing the possible use of the phenomenon as a test for the existence of enteric fever, and the reaction is generally associated with his name. Not only are typhoid bacilli agglutinated by their appropriate serum, but the same phenomenon

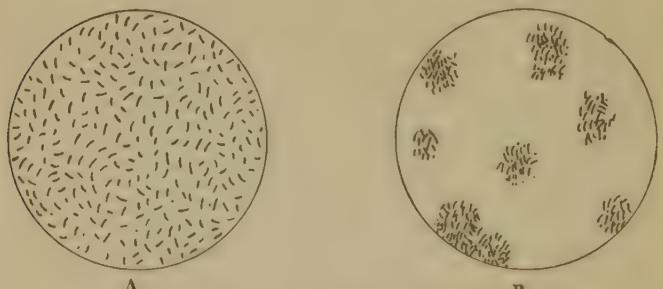

Fig. 1.—Diagrammatic representation of agglutination.
A, Free bacilli; B, Bacilli agglutinated.

occurs with other organisms, such as the *Micrococcus melitensis* of Malta fever, the bacillus of dysentery (Shiga), streptococci, tubercle bacilli, &c.

It was at first thought that the property of agglutinating bacteria was "specific," *i.e.* that a serum would only clump the particular kind of organism which had been injected into the animal, or which had caused disease in the patient. This appears to be only generally true. On the one hand, it is found that a certain degree of agglutinative power towards many different kinds of bacteria may exist in normal blood, so that inoculation or disease only increases an already existing property; on the other hand, it appears that in some cases, at all events, treatment with a particular organism may increase the agglutinative power as affecting other varieties of germs. Thus, in a case quoted by Posselt

and Sagasser,[1] it was found that the serum of a rabbit before treatment possessed an agglutinating power against typhoid bacilli in a dilution of 1 : 10; against colon-bacilli, 1 : 8; against cholera-vibrios, 1 : 10; and against Shiga's bacilli (dysentery), 1 : 5. After inoculation with colon-bacilli, the figures rose to :—*B. typhosus*, 1 : 150; *B. coli*, 1 : 650; *V. cholerœ*, 1 : 50; and *B. dysenteriœ*, 1 : 80. Thus treatment with one organism may apparently increase the agglutinative power against a number of others, and hence the property cannot be looked on as quite specific. It is noticeable, however, that the clumping power towards the bacilli injected rose much more rapidly and to a vastly higher point than that towards other organisms.

Nature of the agglutinative process.—That the agglutinating power depends on a definite substance present in the serum is shown by the fact that it is possible to exhaust the agglutinin in a specimen of serum by adding a sufficient amount of the bacteria on which it acts. Thus if we continually add fresh quantities of typhoid bacilli to the serum derived from a patient suffering from enteric fever, there at length comes a time when no further aggregation of the organisms takes place. But such a specimen of serum may still agglutinate other organisms, as, for instance, *B. dysenteriœ*. This proves that different substances serve as agglutinins for different species of organisms.

The agglutinins are in all probability not the same as the antibacterial bodies by which immunity is brought about, but in the majority of cases they seem to be developed in the serum *pari passu* with the latter. It has been suggested that the agglutinating power might be used as a criterion of the strength of an immune serum. Koch considers that in tuberculosis the agglutinative power possessed by the serum is an index of the patient's power of resistance. It is held by Ruffer and Crendiropoulo[2] that

[1] *Wien. klin. Woch.*, 1903, No. 24, p. 691.

[2] *Brit. Med. Journ.*, April 5, 1902, p. 821.

the agglutinating substances are formed by the leucocytes, especially the multinucleate variety generally associated with inflammation.

The exact method by which the agglutination of bacteria by their appropriate serum is brought about is not understood. It has been suggested that it is owing to some alteration of their covering membrane, so that they are rendered liable to be wetted by the fluid in which they are floating (Defalle[1]). Bodies which are wetted by a liquid in which they are suspended tend to adhere to one another, while those which are not so wetted tend to repel each other.

A more probable explanation is that some proteid substance is precipitated by the action of the serum and binds the bacteria together in its meshes. It is noteworthy that in old cultures of typhoid bacilli an agglutinative substance passes out into the culture-fluid, so that the addition of a portion of such fluid, freed from organisms, confers on a normal serum the power of agglutinating the bacilli. The relation between precipitins and agglutinins is probably very close.

It would appear that two separate bodies take part in the process of agglutination, analogous to the two required for bacteriolysis, hæmolysis, &c. Thus Bail[2] finds that the serum of a patient suffering from enteric fever loses its agglutinative power if heated to 70° C., but that if the bacilli (*B. typhosus*) are kept for some time in this heated serum, the addition of normal serum will cause them to be clumped.

There is some evidence that although agglutination and bacteriolysis are separate processes, yet bacilli are rendered less virulent by agglutination. Besredka[3] found this to be the case with typhoid bacilli, animals being able to withstand larger doses of the clumped organisms than of the

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 595.
[2] *Prager med. Woch.*, 1901, Nos. 32 and 33.
[3] *Ann. de l'Inst. Pasteur*, 1901, p. 207.

normal variety. Bright and Temple [1] had previously noted that the bacilli are maimed, but not killed, by agglutination.

Both agglutinins and bacteriolysins "fall out" with one of the globulin-fractions when serum is precipitated with ammonium sulphate. Thus the typhoid agglutinin falls out with the euglobulin in the serum of goats and rabbits, but with the pseudo-globulin in the serum of the horse. Cholera-agglutinin is precipitated with the euglobulin in the serum of both horse and goat.[2]

Maternal transmission of immunity.—The question of the transmission of immunity from mother to offspring is one of considerable interest, but is not yet satisfactorily elucidated. Agglutinating power is not usually transmitted in the case of enteric fever, nor in tuberculosis; but occasionally such transference is found.[3] Hæmolysins are transmissible in animals (Kreidl and Mandl [4]), and immunity to diphtherial toxine (six out of twelve guinea-pigs, Anderson [5]): so too is supersensibility to horse-serum, according to this last observer. Immune bodies of different kinds may pass into the milk: thus Courmont and Cade [6] found that agglutinins were transferred to an infant through the milk of a wet-nurse, and Salge [7] finds that diphtherial antitoxine may be so transmitted and absorbed by the infant. According to v. Eisler and Sohma [8] opsonins are not transmitted by the mother to the fœtus *in utero*, but they are present in the milk. In all these cases the properties conferred are merely passive and are soon lost by the

[1] *Brit. Med. Journ.*, 1897, i. 206.

[2] Pick, *Hofm. Beitr.*, 1901, p. 384.

[3] Rodhain, *ibid.*, 1903, iii., Hft. 3.

[4] *Wien. klin. Woch.*, 1904, No. 22, p. 611.

[5] *Bull. Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Serv.*, 1906, No. 30.

[6] *C.R. Soc. Biol.*, Nov. 25, 1899.

[7] *Jahrb. f. Kinderheilk.*, 1904, lx. 1.

[8] *Wien. klin. Woch.*, 1908, No. 19.

offspring. The father, as might be expected, has no power of transmission of immunity (Remlinger).[1]

Absorption of toxines and antitoxines by the mouth.—Closely allied to the question of maternal transmission of immunity is that of the absorption of antibodies when administered by the mouth. Hewlett[2] found that diphtherial antitoxine administered by the mouth was useless in protecting animals against the poison of the disease, and this is confirmed by Salge,[3] who finds, however, that the antitoxine present in the mother's milk is absorbed. If this is confirmed, it opens up an interesting question in proteid digestion; for it is usually held that proteins are broken up into their constituent amino-acids and other bodies in the process of digestion and re-synthesised in the system. Diphtherial antitoxine is probably a complex proteid body and suffers the same fate; but it would seem that the proteins in mother's milk, being closely related to or identical with those of the child, may be absorbed intact. Toxines give by the mouth (*e.g.* tuberculin) are said by some observers to be absorbed, but according to Ransom[4] and to Tchitkine[5] the toxines of diphtheria and tetanus do not enter the system by this route. Further experiments are, however, necessary in regard both to this and to other toxines, as McClintock and King[6] state that in rabbits some absorption of antitoxine occurs from the alimentary canal, and that this may also occur in man; while Onorato[7] states that similar absorption takes place in the guinea-pig, the antitoxine being recognisable in the blood within 12 hours, and present for over a fortnight.

[1] *Ann. de l'Inst. Pasteur*, 1899, p. 189. *See also* Merkel, *Münch. med. Woch.*, Feb. 23, 1904, p. 329; Covazza, *Il Policlin.*, Mar. 5, 1903; Bertarelli, *Centralbl. f. Bakt.*, 1906, Orig., xli. 767.

[2] *Lancet*, 1902, i. 375 (*Proc. Path. Soc. Lond.*).

[3] *Op. supra cit.*

[4] *Deut. med. Woch.*, 1898, p. 117.

[5] *Ann. de l'Inst. Pasteur*, 1905, xix. 335.

[6] *Journ. Infect. Dis.*, 1906, iii. 701.

[7] *Ann. Ist. Maragliano*, Genova, 1904, i. 159.

Vaccines (*i.e.* dead bacilli) given by the mouth are said by some observers to exert the same immunising effect as when administered subcutaneously. Here again further confirmation is needed.[1]

We have thus seen that the serum of an "immunised" animal may contain not only antitoxic substances capable of neutralising the poisons of bacteria, and antibacterial bodies (complement and copula) fitted to destroy the organisms, but also substances which agglutinate bacilli or corpuscles, others which destroy living cells (cytolysins), and others still which cause a precipitate with the albumens of the serum of other species (precipitins).

Welsh has suggested that in cases of infection a conflict may be supposed to occur between a bacterium and the body-cells, each side replying to the destructive substances brought against it by its opponent, with antibodies capable of neutralising them—toxine being met by antitoxine, bacteriolysin by antibacteriolysin, and so forth. Enough has been said, at any rate, to show the immense complexity of the serum, and the capacity possessed by animal bodies of protecting themselves in many ways against injurious influences.

Ehrlich's theory of immunity. — Having thus briefly sketched the peculiar properties which are conferred on the blood-serum by treatment with bacteria and other bodies, it remains to consider the theory of the production of immunity and allied phenomena which at present holds the field. This is due to Ehrlich, and is known as his "side-chain" hypothesis. The name is taken from organic chemistry, in which complex molecules have the property of picking up and combining with other atom-groups. Thus in the example given in Fig. 5 we see that a benzene nucleus has joined to itself three NO_2 groups and one OH group, becoming trinitro-benzene or picric acid.

The chemical processes which occur in living protoplasm

[1] *See* Loeffler, *Immunisierung per Os.* Berlin; 1906. Wright, *Lancet*, 1908, ii. 232. Also *infra*, p. 287.

are, of course, much more complicated than those of inorganic matter. Instead of a comparatively simple change brought about once and for all, as in the interaction of two simple salts, or the rather more complex phenomena of organic chemistry, we have a continual series of changes taking place between a mass of protoplasm and the surrounding lymph. The molecule of living matter is itself vastly complex. We know that it can break down into a number of simpler substances, such as albumin, globulin, lecithin, &c., each of which is in reality a complex body, yet all of which are loosely or tightly bound together into

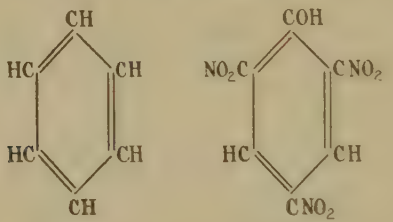

Fig. 5.

a huge molecule of protoplasm. Of the true nature of this last we have no real knowledge. For the purpose, however, of forming a mental picture of the chemical processes taking place in living matter, we may imagine the cell as consisting of a central mass—corresponding with the ring of a benzene molecule—to which are united outlying groups of molecules which have the power of entering into combination with other substances circulating in the lymph, such as particles of food, &c. These outlying groups are the "side-chains" of Ehrlich's theory. Thus a side-chain attached to a cell may join to itself a particle of oxygen, of carbohydrate, of fat, &c., and thereby take part in the nourishment of the cell; or it may become united with a molecule of poison, such as the toxine of the diphtheria-bacillus. In the latter case, either of two things may conceivably happen: the toxine may, through the medium

of the side-chain, become part of the whole cell and may thus poison it, producing actual death (necrosis) or degeneration (*e.g.* cloudy swelling); or it may cause the death only of the individual side-chain to which it has attached itself, in which case the latter is thrown off and a new one is formed by the cell. This reproductive process is supposed to represent what takes place in the presence of only a small quantity of poison, such as first reaches the cell in a case of disease.

The side-chains of living cells, in virtue of their properties of taking up food and other materials—useful and harmful—from the lymph, are known as **receptors.**

By way of illustration of the working of this hypothesis

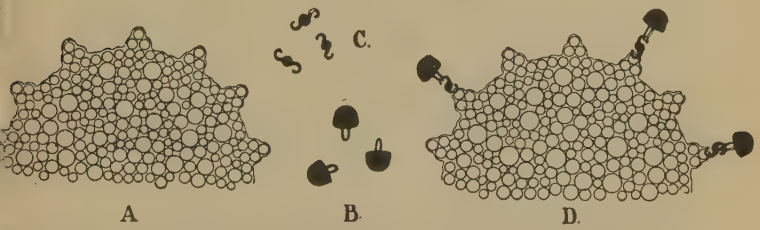

Fig. 6.—Diagrammatic representation of hæmolysis (or cytolysis). A, Corpuscle or cell, consisting of molecules of different sizes, with special groups (side-chains, receptors) at the periphery. B, Molecules of complement. C, Copula or intermediary (immune) body. D, Corpuscle with complement-molecules attached to side-chains by copula.

we may take the process of hæmolysis, as it affords perhaps the easiest example. Here, as we saw above, two substances, (*a*) and (*b*), are necessary to effect destruction of the corpuscle. Ehrlich's theory supposes that the protoplasm of the corpuscle has not the power of combining directly with the dissolving substance (complement) which is always present in serum, but that it can attach to itself a second body (produced in immunised animals), which in its turn can grapple to itself the solvent; and that thus the destructive matter is enabled to combine with the corpuscle and dissolve it. This process is illustrated in Fig. 6.

An exactly similar process is at work in bacteriolysis; the bacteriolysin or ferment present in the serum being attached to the bacteria by a copula or immune body—a side-chain thrown off from the cell.

The process by which a bacterial toxine acts on a cell, though at first sight more direct, is found to be very similar to the action of a hæmolytic substance on a corpuscle; only the toxine consists of both destructive substance and uniting substance joined together in one molecule. The two parts in this case are called respectively the toxophore and the haptophore (τοξικόν, poison; φέρω, I carry; and ἅπτω, I join; φέρω, I carry). The combined toxic molecule seizes on an appropriate side-chain of a cell; and if a number of side-chains thus take up poisonous groups, the cell itself dies. If only one or two side-chains are thus attacked, they are themselves killed and drop off, but the cell escapes. It then proceeds to put out a fresh supply of the particular kind of side-chains, of which some have been killed. As frequently happens in living bodies, the repair goes beyond the original supply, and the cell thus becomes furnished with an increasing number of the side-chains capable of fixing the particular toxine.

But as this process goes on, the cell forms so many side-chains that it cannot keep them all attached to itself, and some of them are cast off into the lymph around the cell and ultimately get into the blood. *These free side-chains constitute the antitoxine.* They are capable of uniting with the molecules of the toxine before it reaches the cells, and in this way they prevent any poisonous action resulting. Further, if the serum containing these free side-chains is injected into another animal, they will still perform the same office under their new conditions, and will confer on the second animal the same immunity as was possessed by the original immunised one. The curative and prophylactic action of antitoxine is thus explained (*see* Fig. 7). If we add to some toxine an equivalent

quantity of antitoxine,[1] the molecules of poison present become combined with the free side-chains in the antitoxine, and can no longer attack the tissue-cells. Hence the injection of a mixture of the two is innocuous. If the bacteria have already gained a footing in the patient and are pouring out constantly a stream of toxine, the injection of a dose of antitoxine neutralises the poison; but it is necessary to give a very large dose of it in order to meet the continuous inflow of toxine. If, on the other hand, a

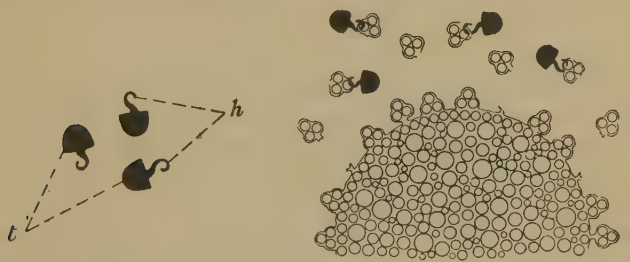

Fig. 7.—Diagram of cell with numerous side-chains (receptors) produced by stimulation with toxine. Some of these have been cast off as antitoxine, and are combining with molecules of toxine. On the left are seen molecules of toxine, showing *t*, toxophore, and *h*, haptophore, elements.

person has not yet got, say, diphtheria, but is exposed to the chance of infection, then a protective dose of antitoxine may be given to anticipate infection, so as to be lying in wait, as it were, for any poison that may be formed.

To this last application it might be objected that, as previously stated, the antitoxine is not antibacterial, and that, therefore, it does nothing to stop infection. But as the bacilli conduct their conflict with the body by means of their poisons, injuring the cells and so preventing them from

[1] Properly speaking, antitoxine is the actual substance which combines with the toxine and neutralises it. In ordinary parlance the word is used for the serum containing the antitoxic body. For further consideration of the interaction of toxine and antitoxine, *see* p. 73.

forming antibacterial matter, the neutralisation of the first doses of poison enables the organism (the animal attacked) to gain time to form its defensive weapons. To use a military simile, we may do much to resist the first assault if we can damp the powder of our antagonists, and so we may enable our own reinforcements to come into action.

It is important to remember that by administering antitoxine we only neutralise the *free* toxine present. That which has already entered into combination with the side-chains of the tissue-cells is practically beyond the reach of the remedy. It is possible that, if there is a very large quantity of antitoxine present, some kind of "mass" action may take place, whereby the toxine may be withdrawn from its combination with the cells and caused to combine with the antitoxine instead; but this is not absolutely certain. Hence arises the urgent need, in giving antitoxine, to give it as early as possible in the disease. For example, in the case of tetanus, it may be too late to make use of antitoxic serum when the symptoms of the disease have appeared, the poison being already closely attached to the cells. Hence many failures and much disappointment in the treatment of this disease.

It may be pointed out that so long as the side-chains are attached to the cells they are a source of weakness, as enabling the toxines to attack them, whereas, when they are cast off into the serum as antitoxine, they become a protection. A toxine can only act if it finds appropriate side-chains to which it can attach itself; otherwise it would circulate harmlessly in the lymph. Since it would be better for the cell, from the point of view merely of its relation with toxines, if it had no side-chains, it is supposed that these exist originally for some other purpose; and this is considered to be the assimilation of food-materials, as already suggested. These presumably are taken up by the cell for the purposes of nutrition, in the same manner that toxines are attached, by means of the affinities possessed by the side-chains.

A distinction is held to exist between the poisons formed by bacteria on the one hand—and with them must be grouped the toxines elaborated by poisonous snakes, and those resident in certain plants, such as ricin, abrin, &c.—and the ordinary mineral and vegetable poisons—mercury, arsenic, strychnine, morphine, and the like. It is supposed that the substances which form the first group are proteid in character (globulins, &c.), and that it is towards them especially that the side-chain activity of the cell is directed. The poisons of the second group appear to act directly on the whole cell, as in the case of these substances no antitoxic serum can be prepared. The cell as a whole is either killed or recovers from the effects of the poison, but it cannot protect itself by throwing off side-chains to neutralise the poison. This property is distinct from the power which the body undoubtedly possesses of accustoming itself to increasing doses of poison such as morphine or arsenic.

Incubation-period.—A peculiar feature in the action of bacterial toxines is the occurrence of an incubation-period between the administration of the dose and the onset of symptoms. Thus, by giving increasing doses of tetanus-toxine, the rapidity of the onset of spasm may be increased up to a certain point; but after this is reached, no further addition of poison will accelerate the event. It may be suggested that time is needed, not only for the combination of the toxine with the side-chains, which is probably a somewhat slow process, but also for the absorption of the poisoned receptor into the general body of the cell, which must precede symptoms of intoxication.

Before leaving this subject it may be well to emphasise the fact that the explanation given by Ehrlich of the phenomena of bacteriolysis, the action of toxines, &c., is pure hypothesis. The hypothesis has been fruitful, suggesting new lines of research; and so far the results obtained are mainly consistent with the theory. But care must be taken not to confuse the fascinating diagrams, by which we are enabled to form a mental picture of the events that

take place, with realities. An antitoxine is probably analogous to a secretion, and the process of bacteriolysis is a chemical reaction into which three bodies enter—very similar to the interaction of the fibrin-ferment, fibrinogen, and calcium salts to form actual fibrin. In the present account the illustrations have been kept as diagrammatic as possible, at the expense of verisimilitude and artistic merit, in order to avoid any undue pretence of reality.

Forms of immunity.—We are now in a position to explain, on the theories just set forth, the various forms of immunity already alluded to. In the case of species, races, or individuals who are *naturally* immune to certain infections, we must suppose either that they possess no side-chains capable of uniting with the toxines of the bacterium causing the disease, the latter thus becoming harmless,[1] or that they normally contain in their systems the two substances necessary to repel the bacteria, viz. the complement and the copula. In those who are *artificially* immunised or who have recovered from a disease (*acquired immunity*) the copula has been produced, as has been described, by gradual education of the cells to throw it off.[2] In all these cases the immunity is said to be "active." When an animal has received into its system a dose of antibacterial or antitoxic serum and is thereby enabled to resist a disease, it is said to possess "passive" immunity. This lasts only as long as the injected serum remains in the body; and this is not, as a rule, for any long period of time, since the foreign serum is more or less rapidly excreted.[3] The animal in this latter

[1] An instance of this condition may be seen in the tortoise, which is immune to the toxines of tetanus; if, however, the blood of a tortoise which has received a dose of tetanus-toxine be injected into another animal, it will cause death, showing that the poison is not neutralised in any way, but merely has no power of affecting the cells of the tortoise.

[2] The copula must be of the nature of a special kind of side-chain.

[3] The antitoxine or copula injected may perhaps be neutralised by the formation of an anti-antitoxine or anti-copula, and not merely passed out of the body.

case has not gained for itself any power of forming protective substances; whereas in active immunity its cells have been educated to perform this duty, and this acquirement seems to be retained either permanently or for a considerable period of time. It is found that, if an individual has gained this active immunity to one disease and then becomes infected with a second distinct malady, the former protective power is often lost.

Modification of phenomena of bacteriolysis, &c., in the presence of living tissues.—It might seem from a consideration of the foregoing facts that the explanation of immunity was a fairly simple one—that defence against the invasion of any micro-organism depended on the existence in the blood of the individual of two special bodies, a ferment and an intermediary body capable of joining the ferment on to the bacterium. The absence of either of these would constitute *susceptibility;* their simultaneous presence would confer *protection.* But other ascertained facts render the question more complicated. Thus, taking the case of anthrax-bacilli, we find that the blood of the rabbit, a highly susceptible animal, acts destructively upon the organisms in a test-tube: within the body it manifestly does not do so. Similarly, all white rats possess serum which destroys anthrax-bacilli, but they are not all immune. On the other hand, the serum of animals immune to the disease, as that of the hen, forms a good culture-medium for the bacillus. It thus becomes clear that we have to take into account not only the blood, but also the living tissues by which it is surrounded in the body. In the one case the tissues seem to exercise some inhibitory influence over the bacteriolytic action of the blood; in the other, they supply some factor necessary for the defence of the animal against the bacilli. Further experiments show that while the serum of the rabbit is destructive for anthrax-bacilli, an extract of its organs prepared by triturating them with salt-solution has no such power. Indeed, if crushed organs are added to the serum and the bacilli exposed to the action of

the mixture, no bacteriolysis takes place. The tissue-cells have in some way deprived the serum of its bacteriolytic property.

The explanation[1] given of these phenomena is as follows :—We have already seen that a copula, or immune body, is needed to fasten the ferment to the bacteria and so produce their destruction. This copula has affinity for the tissue-cells—in this case an even greater affinity than it has for the bacilli. Hence it unites with the cells, and is no longer available for the process of bacteriolysis.

Source of complements.—With regard to the source of the complement or alexine, an experiment has been performed which points to the leucocytes as at least one source of these bodies. Thus, dog's serum alone is found not to act bacteriolytically on anthrax-bacilli, but if some leucocytes from the same animal are added to the serum, then destruction of the bacilli takes place. From other data it is believed that the serum of dogs contains the immune body, so that it appears that in this case the leucocytes are the source of the complement (Briscoe).[2] In other cases it has been possible to supply the complement by the addition of an extract of the tissues, so that these also must be regarded as forming ferments capable of acting bacteriolytically in the presence of a suitable copula.

Plurality of complements.—Considerable controversy has centred round the question whether the complements or alexines, by which, for example, bacteriolysis is brought about, are the same for all micro-organisms, and the same in all species of animals. Thus, it might be possible to prepare an immune serum (one containing a copula or intermediary body) which should be capable of immunising a certain species of animal (A) against a particular bacillus, but this intermediary body might only be capable of uniting to the bacilli a special form of

[1] Bail, *Prager med. Woch.*, Nov. 25, 1903, p. 307.

[2] Professor Orth's "Festschrift," Berlin, 1903 (Aug. Hirschwald), p. 396 et seq.; see *Brit. Med. Journ. Epit.*, June 27, 1903, p. 104.

complement such as exists in the kind of animal (A) used. We cannot be certain without making the actual experiment that it will act in the same manner within the body of a second species of animal (B): it may be incapable of uniting with the form of complement which is here present. For example, we might prepare an antityphoid serum capable of protecting an animal (say, horse) against typhoid-bacilli, *i.e.* of causing destruction of *B. typhosus* when it is injected into this animal; but it does not necessarily follow that it will act as a cure in cases of enteric fever in man, since human beings may not possess the kind of complement with which horse-copula can unite so as to attack the micro-organisms.

It has also been a moot point whether in a single species of animal there is present only one complement, which is ready to act upon all bacteria alike, and upon blood corpuscles, cells, &c., if it is only supplied with the requisite intermediary body; or whether more than one complement is present in the serum—one, perhaps, capable of producing hæmolysis, another of causing bacteriolysis. The mode of experimenting is by "preparing" (p. 10) bacteria and corpuscles with a certain immune body, and then adding these prepared bacteria or corpuscles to a specimen of serum till no more lysis takes place. When the serum has thus been saturated with one kind of organism or corpuscles till it can dissolve no more, a second variety of prepared body (corpuscle or bacteria) is added, and it is seen whether destruction of any of this occurs. Results appear to be contradictory. Bordet and Büchner hold to the unity of the complement; Metchnikoff, Neisser, Ehrlich, and Morgenroth support a plurality.[1] It is quite possible that the same answer to the question may not hold good in all species of animals. There is considerable evidence, however, pointing to the fact that—at all events, in some instances—the complement

[1] See *Ann. de l'Inst. Pasteur*, 1899, 1900; *Berlin. klin. Woch.*, 1899, 1900, 1901.

which causes hæmolysis may be different from that causing bacteriolysis in the same animal. Neisser[1] has shown that rabbit's serum can be deprived of its bacteriolytic complement by addition of anthrax-bacilli, but that it still remains capable of hæmolysis. Gengou and Tarassevitch[2] have adduced experiments tending to show that different kinds of leucocytes are the sources of hæmolytic and bacteriolytic complements respectively—the "microphages" producing bacteriolysins, the "macrophages" hæmolysins.

Forster[3] finds that the same complement in goat's serum acts against the organisms both of cholera and of typhoid.

Dangers of excess of copula or complement.—It has been suggested[4] that the presence of excess of antitoxic serum may have an ill effect, since the antitoxine which is not employed in neutralising toxine might give rise to the formation of anti-antitoxine, which would prevent the action of antitoxine in the future stages of the disease. It seems doubtful, however, if this constitutes a practical danger in therapeutics; and considering the entire ignorance which exists with regard to the exact quantity of toxine present in any given case, we must continue to be guided by purely empirical rules for administration of antitoxines. A danger similar to that just mentioned is said to exist in the case of antibacterial serums. If an excess of such a serum be administered, there is produced an excess of copula in the absence of sufficient complement. When the former unites with the bacteria, its affinity for the complement appears to be reduced; at all events, it is not increased. The free copula (the excess) then appears to attach itself to the available complement, and we have bacteria with copula attached to them, and complement-molecules with

[1] *Deut. med. Woch.*, 1900.
[2] *Ann. de l'Inst. Pasteur*, 1900, 1901.
[3] *Lancet*, 1905, ii. 1531.
[4] *See* Ainley Walker, *Clin. Journ.*, June 17, 1903, p. 144.

copula attached to them. This double combination seems to prevent bacteriolysis, as it would be necessary, in order that this should occur, for copula to unite with copula, which is not possible.

Deficiency of complement.—So far we have paid perhaps more attention to the copula than to the complement in the production of immunity. But susceptibility to disease may depend on lack of sufficient complement as much as on defect of the intermediary. Some individuals may be naturally ill supplied with complement. In others pre-existing disease may exhaust the supply. Thus it has been shown that in chronic maladies (carcinoma, Bright's disease, &c.) the quantity of complement present in the serum tends to fall, and in this way we may explain the tendency to terminal acute infections in these conditions. Excessive exertion may perhaps cause destruction of complement, and thus predispose to infectious diseases. The use of such remedies as yeast and cinnamic acid may lie in their power of supplying complements, the former perhaps directly, the latter by stimulating leucocytosis—the leucocytes being the main source of complement.

Fixation or deviation of complement.—As already mentioned (p. 8) if a hæmolytic serum be heated, so as to destroy the complement present ("inactivated"), and be then brought into contact with appropriate blood-corpuscles, the copula present attaches itself to these (Fig. 8, A), and hæmolysis will result if a supply of complement is provided by contact with fresh serum. (It is found that the complement may in many instances be provided by the serum of quite a different species of animal: thus with a mixture of heated hæmolytic rabbit's serum and corresponding ox-corpuscles, hæmolysis will result on addition of goat's serum.) If, on the other hand, the complement and the copula be left in contact with one another (Fig. 8, B), no union results: consequently in such a mixture as the latter (B) the complement is still free, and will bring about hæmolysis if the second mixture is added to the first (A) in which

there is copula along with corpuscles. If, however, to the second mixture (B = complement + copula) some appropriate corpuscles be added, hæmolysis ensues (Fig. 8, C): the complement is used up in the process and is no longer free to produce further hæmolysis in mixture A. A means of testing for the presence of the appropriate corpuscles is thus provided; for if a liquid containing appropriate corpuscles is added to mixture B, its complement is fixed and hæmolysis occurs; whereas if the corpuscles added are

A. Heated hæmolytic rabbit-serum and ox-corpuscles (Copula attached to corpuscles)
B. Goat-serum and heated hæmolytic rabbit-serum (Free complement and copula)
C. (B + antigen) Addition of ox-corpuscles to B produces fixation of complement.

Fig. 8.

inappropriate (belonging to some other species of animal than that used for the original preparation of the hæmolytic serum), no fixation occurs and the complement remains free —consequently no hæmolysis takes place. Since not only blood-corpuscles, but many other albuminous bodies, when injected into animals of a different species, produce antibodies (copulas) which by union with the existing complement are able to combine with the antigen [1] originally

[1] The term "antigen" has been coined to denote any substance which, when injected into a living animal, causes the formation of a specific antibody. Thus bacterial toxines give rise to antitoxines, albumins to precipitins, blood-corpuscles to hæmolysins, and so forth.

injected, the test may be applied to detect the presence of any specific antibody.

Under this heading may be placed the reactions associated with the names of Gengou (precipitation), Neisser and Wechsberg (bacteriolysis), and Wassermann (syphilis). Thus, if a mixture be made of syphilitic antigen, represented by an extract of syphilitic fœtal liver, syphilitic antibody obtained by inactivating the serum from a case of syphilis, and complement, these three bodies become linked together, and the addition of the mixture to sensitised red corpuscles (as for instance in mixture A, Fig. 8) is not followed by hæmolysis, as the complement is deviated from the hæmolytic system to the syphilis "antigen-antibody" combination.

Anaphylaxis.—When the serum of one species of animal is injected into an individual of another species, antibodies are formed as has already been described. But in addition to this formation of antibodies, or as a part of the process, changes of a nature which is not understood take place in the injected animal, by which it is rendered peculiarly sensitive to a further injection of the same kind of serum. Symptoms of severe constitutional disturbance, and sometimes even death, may ensue upon such a second injection. Thus, if a guinea-pig receives an intraperitoneal injection of horse-serum, it suffers no ill effects; but if the injection is repeated in about twelve days' time, it becomes seriously ill. The phenomenon is called "anaphylaxis" (supersensibility or hypersensibility). This condition does not develop immediately, but only after the lapse of a definite interval varying with the serum employed and the animal inoculated. It passes off gradually in a month or more. If in the interval between the first injection and the appearance of anaphylaxis another injection of the serum in question be given, protection against the subsequent development of anaphylaxis is produced (*anti-anaphylaxis*, Richet). Apparently the increased susceptibility is

connected in some way with the formation of protective antibodies, of which Richet considers it to constitute the first stage. Milk, egg-albumen, bacterial proteins and other substances are all capable of inducing anaphylaxis.[1] The condition is of interest as an explanation of many cases in which ill effects have followed the use of antitoxic serum (*see* p. 58). Besredka[2] finds that heating the horse-serum deprives it of toxic power, and that if the animal is anæsthetised at the time of the second injection, no ill effects result.

Opsonins.—French observers, studying the phenomena of phagocytosis under the influence of Metchnikoff, early discovered that the activity of the leucocytes was greater in the presence of serum than without it (Denys and Leclef). This was confirmed by Mennes and others. The influence of the serum has been specially studied by Wright and his collaborators in recent years, the name "opsonins" having been coined for the chemical substances at work (Wright and Douglas).[3] Increase in the rapidity of phagocytosis might theoretically be brought about either by an increase in the voracity of the leucocytes or by a diminution in the resistance offered by the bacteria to their attack, and both processes have been supposed to occur. The existence of substances which increase the activity of the leucocytes ("*stimulins*," Metchnikoff, Leishman) is now doubted by most observers, the action of opsonins being to render the bacteria either more attractive (chemiotactic) or less resistant to the phagocytic cells. Some doubt has existed as to the possibility of phagocytosis in the absence of serum, but it seems to be established that it does occur, though only to a slight degree. The presence of normal serum increases the activity of the

[1] Rosenau and Anderson, *Bull. Pub. Health and Mar. Hosp. Serv. U.S.A.*, 1907, No. 30; 1906, No. 29; and 1909, No. 50.

[2] *C.R. Soc. Biol.*, 1907, lxii. 1053. *See* Richet, *Ann. de l'Inst. Pasteur*, 1907, July, p. 497.

[3] *Proc. R. Soc. Lond.*, 1903, lxxii. 357; 1904, lxxiii. 128.

leucocytes in ingesting all kinds of bacteria; and when infection with a pathogenic organism has occurred and been successfully resisted, the opsonic power of the serum is found to be increased towards that particular organism. It appears that opsonins formed in response to the stimulus of an infective organism are strictly specific. There seems reason to hold that the opsonin of normal serum is distinct from those of "immune" serum, inasmuch as the former is destroyed by heating the serum to 65° C. (thermolabile), while the latter are thermostable. The opsonic power of a serum rapidly diminishes when it is kept at room-temperature, Knorr [1] finding that it is reduced by one half in twenty-four hours and entirely abolished in five days.

The exact nature of opsonins and their relation to other substances concerned in immunity are doubtful. Thus Grüber and Futaki [2] regard them as complements, owing to the destruction of normal opsonins by heat. The converse of this argument is used to support the view that "immune" opsonins are really copulas (amboceptors), since they resist a temperature of 60° C. Greig Smith [3] holds that they are identical with agglutinins. Verney [4] believes that all varieties of immune substances — complements, copulas, antitoxines, and so forth—can act as opsonins. The existence in opsonins of two groups, analogous to the haptophore and toxophore groups of a toxine, is maintained by Hektoen [5] and Kurt Meyer.[6] Seeing that in a mixture consisting of serum, leucocytes, and virulent and saprophytic bacteria the leucocytes ingest the saprophytes much more readily than the parasites, it is reasonable to suggest that the opsonin acts by neutralising the toxine or "aggressin" (Bail) by which the pathogenic germ repels the phagocyte.

[1] *Journ. Amer. Med. Assoc.*, 1907, xlviii., No. 15.
[2] *Münch. med. Woch.*, 1906, liii. 249.
[3] *Proc. Linnean Soc. N.S.W.*, 1905.
[4] *Policlin.*, 1907, Sec. Pract., No. 40.
[5] *Journ. Amer. Med. Assoc.*, 1906, xlvi., No. 19.
[6] *Berlin. klin. Woch.*, 1908, p. 951.

But the question is at present unsettled. Some chemical substances, such as formalin, chloroform, and alcohol, act as "anti-opsonins," diminishing phagocytic activity (Hektoen). Anti-opsonins are also said to be formed in cultures of bacteria (Tchistovitch and Jurievitch)[1]: these latter are probably toxines or aggressins.

Method of estimating the opsonin-content of the blood-serum.—For this estimation it is necessary first to prepare a quantity of living human leucocytes from some indifferent source (either the patient or some normal individual), from which the plasma has been completely removed. This is done by receiving the blood, as it exudes from a needle-puncture, into a weak solution of sodium citrate, centrifugalising the mixture thoroughly, pipetting off the citrated

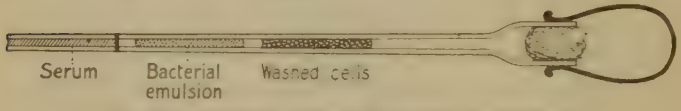

Fig. 9. Measuring pipette for opsonin-estimation.

plasma, adding normal saline solution to the deposited mass of red cells and leucocytes, mixing thoroughly and again centrifugalising. A repetition of this "washing" with normal saline and the subsequent removal of the supernatant fluid leaves a sufficient quantity of leucocytes and cells entirely freed from the fluid (which possibly contained excess of opsonin) in which they were originally suspended.

Next a small quantity of the blood to be tested is collected in a small pipette and allowed to clot, and the serum is separated. Similarly the serum from the normal individual or "control" is prepared. Finally a homogeneous suspension of the test-bacterium is prepared by emulsifying some of the bacterial growth in distilled water and carefully centrifugalising, in order to throw down any possible clumps or masses of micro-organisms. By means of a suitable measuring pipette (Fig. 9), equal

[1] *Rousski Vratch*, 1908, vii. 669.

quantities of washed cells, of bacterial emulsion and of the patient's serum are taken up and thoroughly mixed, and the mixture incubated at body-temperature for fifteen minutes. At the end of this time, after preliminary manipulations to ensure that there is thorough incorporation of the mixture, blood-films are spread therefrom on an ordinary glass slide, fixed, and stained. A second mixture, in which the normal serum is substituted for the patient's serum, is prepared in an identical manner. The two slides are examined microscopically, and the

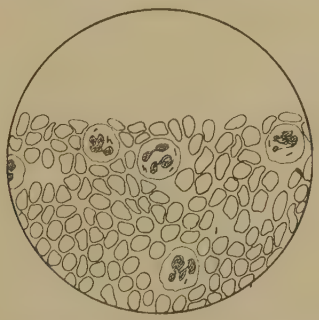

Fig. 10.—Edge of blood-film preparation, showing phagocytosis of bacilli by polymorphonuclear leucocytes in the presence of serum containing opsonin.

number of bacteria taken up and ingested by the first fifty consecutive polymorphonuclear leucocytes encountered in each film is noted (Fig. 10). The ratio between the resulting sums is then expressed in the form of a fraction, of which the total contents of the fifty cells mixed with the patient's serum forms the numerator, and that of those with the normal serum the denominator. This fraction is, however, usually expressed as a percentage of unity—which is represented by the normal serum. Thus, supposing 150 bacteria were counted in fifty cells of the preparation made from the patient's serum, and 100 in a similar number in the preparation from the normal control, the index would

be represented by $\frac{150}{100}$ or 1·5. If, however, the specimens chosen had given the figures 60 and 90 respectively, the fraction would have been $\frac{60}{90} = 0{\cdot}66$.

Value of opsonic determinations.—The accuracy of opsonic determinations as usually practised depends on the postulates that all polymorphonuclear leucocytes, whatever their source, are equally active in ingesting bacteria, and that the opsonic power of the serum is constant in normal healthy persons. The former supposition is contrary to what obtains in the case of other vital phenomena, in all of which differences are found to exist between individuals; and the uniformity of leucocytic action has been questioned on experimental grounds by several observers (Ledingham,[1] Rosenau,[2] Potter[3]). Many series of experiments have been performed to elucidate the constancy of the opsonic content of normal persons. Bulloch[4] found that the tubercular opsonic index in healthy persons might vary between the extremes of 1·2 and 0·8, the majority of individuals being practically equal (1·0). Such a difference seems small in itself, but it is clear that a considerable percentage of error may be introduced thereby into individual calculations. Thus, if a normal serum with an index of 1·2 be taken as the standard, the normal serum at the other extreme of the scale will be found to have an index of 0·66; while conversely, if the lower normal index be taken for comparison, the higher will show an index of 1·5. Both 0·66 and 1·5 would be held to be pathological and to point, the former to the possibility and the latter to a probability, amounting for practical purposes to a certainty, of tubercular infection.

This difficulty is, however, overcome in practice in one

[1] *Journ. Infect. Dis.*, vol. iii. 683.

[2] Quoted by Dean, *Brit. Med. Journ.*, 1907, ii. 1409 (with full literature).

[3] *Journ. Amer. Med. Assoc.*, 1907, xlix., No. 22.

[4] *Lancet*, Dec. 2, 1905.

of two ways, either by using as the normal serum the "pooled" serum of several normal individuals, or by always taking the serum from the same healthy individual as the normal; for the index of the normal serum, whether at one limit or the other of the normal range, remains practically stationary from day to day.

More serious even are the doubts that have been thrown on the accuracy of the ordinary method of estimating phagocytic activity. It has been shown by Fitzgerald, Whiteman and Strangeways,[1] that different workers may with the same serum obtain widely divergent results. For example, the following pairs of figures give extreme instances of such divergence, each pair representing the indices obtained from one specimen by different observers:— 1·70–0·36, 1·92–0·68, 1·18–0·31. Further, if, on the same slide, successive series of 50 leucocytes are counted, very different numbers of enclosed bacilli are found. Thus, on the same slide in one instance, one series of 50 cells contained 119 bacilli, and a second series of 50 contained 74 only. In another instance the numbers were 150 and 71. Even when 100 cells were counted instead of 50, the percentage difference amounted in one instance to 115. Hence, these writers conclude that for accuracy of observation not less than 1,000 cells must be counted. Since this would involve an expenditure of time amounting to several hours for each slide, it is clear that, if these writers are correct, accurate estimations are beyond the sphere of practical use. Greenwood,[2] however, considers that counting 1,000 cells will not necessarily give greater accuracy than counting 25.

Even admitting that accurate estimations of the phagocytic activity of a serum can be made, it remains to consider what the value of such an estimation is as a measure of a patient's degree of immunity. It is admitted,

[1] *Bull. of the Committee for the Study of Special Diseases.* Cambridge, 1907, i., No. 8.

[2] *Biometrica.* Cambr., 1909, 6, Part iv.

even by Wright himself, that the opsonins are not the sole means of resistance to bacteria; there are besides, not only the agglutinins, bactericidins and antitoxines, but also the resistance of the cells of the various tissues, which probably plays a very important part. Measurement of the opsonic power of the serum therefore throws light on only one factor in immunity. In earlier days the agglutinating power of the serum was used as an index of immunity, until time showed that it was untrustworthy. Similarly it cannot be accepted without more rigorous proof, that the amount of opsonins present is a fair measure of the total resistance. In many instances the variation of the opsonic index does not correspond with the clinical course of a case, while it is known to alter in response to slight influences which cannot be supposed to affect the general degree of resistance. Thus, Latham[1] found that the opsonic index in tuberculous individuals varied throughout the day inversely with the patient's temperature, while a slight degree of bodily exercise in such a patient may notably reduce the index.

In spite of these theoretical objections, many competent observers believe that the opsonic index is of considerable value in indicating the general trend of the process of immunisation. Moreover, there can be no doubt that the study of the phagocytic properties of the serum has not only opened up a most interesting chapter in pathology, but has had at least one very important and most beneficial result, in that it has led to a great reduction in both the frequency and the size of the doses of tuberculin generally employed (*see* p. 286). The effects of minute doses are now recognised, and a great step towards a rational use of the remedy has been taken.

[1] *Proc. Roy. Soc. Med.*, i., 1908, Med., p. 195.

CHAPTER II

PREPARATION AND ADMINISTRATION OF SERUMS AND VACCINES

SERUMS

Preparation of antitoxic serum.—Some account of the preparation of individual serums will be found under their separate headings; only a general outline of the processes adopted will here be given. It is necessary, first of all, to make sure that the animal (generally a horse) to be inoculated is itself free from disease of any kind which might be transmitted to human beings. For this purpose it is submitted to a preliminary test by being injected with tuberculin to eliminate the presence of tuberculosis, and with mallein to ensure the absence of glanders. If it prove sound, it is in some cases first inoculated with a dose of attenuated (weakened) toxine, prepared by heating the virulent poison, or by treating it with some chemical agent which reduces its strength. After this the animal is inoculated with increasing doses of the virulent poison at stated intervals of time. Each dose is generally sufficient to produce some febrile reaction, from which the horse recovers in the intervals. The doses are given, as a rule, subcutaneously, but they may finally be administered intravenously when a high degree of immunity has been attained. Sometimes, as a finishing touch, the bodies of the actual dead organisms are injected. The horses used for the preparation of serum generally flourish under the treatment, and grow sleek and fat. They are liable, however, after a time to develop symptoms of disease; amyloid change may

occur in the liver and spleen, and a fatal hæmorrhagic hepatitis may sometimes ensue.[1]

The toxines used for injection are prepared by growing the organisms in suitable fluid media; the cultures are then passed through a Pasteur-Chamberland or other similar filter of porcelain to remove the bodies of the bacteria. It is important that the toxines should be as potent as possible, and special methods are adopted to secure the highest possible degree of virulence.

As the site for the injections administered to the horse the root of the neck is generally selected, the hair being first shaved, and the skin thoroughly scrubbed with lysol or some other antiseptic. When a sufficient degree of immunity has been reached, the blood is withdrawn from the jugular vein by means of a simple incision, made with all antiseptic precautions, a sterile cannula being thrust into the vein, and the blood received into sterilised vessels. In these it remains till coagulation has taken place, and the free serum is then decanted off and mixed with a small quantity of some antiseptic. It is transferred to bottles of convenient size, and is ready for use. A large quantity of blood can be obtained at a single operation from a horse (16 to 20 pints from a good-sized animal) without ill effects. It is important to wait for a few days after the last injection of toxine before withdrawing the blood, as otherwise the serum may contain poisonous material.

Antibacterial serum.—Antibacterial serums are produced by very similar means, but the actual bacteria are injected instead of their toxines. Sometimes the dead bodies of the organisms are first used, or an attenuated culture, the virulent bacteria being withheld till some degree of tolerance is established. A dose of antitoxic serum may be used to mitigate the effects of the first dose, if it be available. In the case of antibacterial serums it is most important that the preparation used for treatment of

[1] Lewis, *Journ. of Med. Research*, 1906, xv. 449.

disease should be freshly made, since it has been shown that the value of such serums rapidly falls.

Two special factors enter into the question of the manufacture of antibacterial serums which do not apply to antitoxic preparations. In the first place it is found that some species of bacteria comprise different strains or varieties, which react differently towards protective serums. Thus, a serum may be prepared which will be fatal to a certain strain of streptococci—the variety used for its preparation —but which will have no similar effect on another race of the same organisms, derived perhaps from a different patient or from a slightly different form of pyogenic disease. Now, in any individual case of illness we cannot tell what strain of bacteria may be present, and therefore in preparing a serum for practical therapeutics it is advisable to use several strains for the purpose of immunising the animal, in order that the chances of combating the causal organism in any human patient may be increased. A serum thus prepared with several strains of an organism is said to be "polyvalent."

Again, as has already been pointed out, it does not follow that the complement existing in the body of one animal will be capable of uniting with the copula supplied by another animal, so as to destroy a given bacterium. An antibacterial serum originally, no doubt, possesses in itself both complement and intermediary body. But the former is an unstable substance : it rapidly vanishes from the serum when it is kept,[1] and it is not improbable that it is destroyed when the serum is injected into another kind of animal. Hence the copula contained in the immune serum may have to depend on a complement found in the animal or man to which it is administered, in order to have a bactericidal effect.

If the two bodies do not fit one another, no curative result will ensue. It has been recommended, therefore, that serums for human use should be prepared from some

[1] *See* Ainley Walker, *Lancet*, 1901, i. 18.

animal nearly allied to man, such as the ape. Such a serum is said to be "homologous."

In the case of many antibacterial serums—for example, those prepared against streptococci and pneumococci—the presence of leucocytes seems necessary to the action of the serum. Apparently it is an opsonising influence that is exerted rather than a true bacteriolysis.

Testing serum.—Before a specimen of serum is issued for use, it ought to be tested, to ensure that it is free from contamination. It must not contain living bacteria or toxines. In order to test it, a portion of it must be mixed with some culture-medium and incubated, to see if any bacteria develop. Some of it must also be injected into an animal to make sure that it is not toxic. Cases have occurred in which death has been caused by the use of a serum containing the toxines of tetanus.

Standardisation of serums.—Since it has hitherto been found impossible to isolate the actual toxines of bacteria, so that no process of weighing or measuring can be applied to them, it is necessary to devise some other way of calculating their strength. A physiological test of some sort is the only available means of measuring their effects. For this purpose it is necessary to find some animal which reacts in a constant manner to the poison, dying regularly within a certain time as the result of a given dose of toxine. In the case of the diphtherial toxines it is found that guinea-pigs are suitable test-animals. It is possible to ascertain accurately the quantity of a particular specimen of poison which will invariably cause the death of a guinea-pig of a certain weight (250 grammes) on the fourth day. This is taken as the standard dose of poison, or "minimal lethal dose." It is then necessary to find what amount of antitoxine is required to neutralise this dose exactly, and we find that equal quantities of a given antitoxine will perform this duty. A standard is thus set up. What is known as a "unit" of antitoxine is that quantity of antitoxic serum which will exactly neutralise 100 minimum lethal doses as

above described, *i.e.* of which one hundredth part will prevent the appearance of any morbid symptoms in a guinea-pig, if injected along with the minimal dose of toxine which would otherwise kill it within four days.

In the case of an antibacterial serum the matter is rather more complicated. Take as an example a serum which destroys cholera-vibrios. Such serum, if injected into the peritoneal cavity of a normal animal along with a loopful of virulent cholera-organisms, will rapidly cause their disintegration. On the other hand, the bacteria, if injected into the abdomen of an animal (not immune) without any protective serum, rapidly multiply and kill the animal. In testing the strength of a serum different portions are diluted for the sake of accuracy, to (say) 1 : 100 and 1 : 1000. Two guinea-pigs are taken, one of which receives 1 cc. of the first dilution along with a loopful of a virulent culture of cholera-vibrios, while the other receives the same quantity of the second dilution with the loopful of the bacteria. Within forty minutes search is made in the peritoneal cavities of the animals to see whether the vibrios therein are flourishing or are disintegrating. If the smaller dose of serum has failed to kill them, while the larger one has done so, further experiments are made to determine the exact quantity of serum which just suffices for the purpose; if the lower dose has proved sufficient, then smaller quantities still are tried, and that dilution of the serum which is finally determined (*e.g.* 1 : 500) is used to indicate the valency of the anti-serum.

VACCINES

Methods of vaccination.—To confer active immunity, it is necessary to inject either the actual bacteria or their toxines into the individual to be immunised. For the purpose of vaccination it is necessary to inject a dose of the bacteria which the individual is capable of overcoming by means of his natural powers of resistance, the result being to raise his protective properties against future

infection by stimulating the tissue-cells to manufacture anti-bacterial bodies. It is evident that to inoculate a person or animal with virulent bacteria by the same channel as that by which infection is produced in disease would merely induce the very condition from which it is sought to gain protection. Some other method must be selected. Several different ways of inoculation without conveying an actual attack of the disease are available :—

1.—It is possible to inoculate attenuated organisms, *i.e.* bacteria which have lost some of their power of producing disease. Attenuation[1] may be brought about (*a*) by passage of the organisms through an animal which has a greater power of resistance to them than man, as in the case of small-pox, which in the cow takes the form of the mild disorder, vaccinia ; (*b*) by passage through an animal which, although it is as sensitive as man or even more so, yet alters in some way the properties of the bacteria, so that they are less adapted to cause human infection : thus it is said that the virus of hydrophobia, after passage through a series of rabbits, although its virulence for these animals is immensely increased, is yet rendered less potent for mankind ; (*c*) by growing the germs under conditions unfavourable to their development : anthrax-bacilli grown at a temperature of 40° C. lose much of their virulence, and Pasteur made use of this method for preparing a vaccine for the protection of animals against this disease ; (*d*) by addition, to cultures of the organisms, of chemical substances inimical to their growth : thus tetanus-bacilli may be attenuated by means of iodine trichloride for the purpose of the first inoculations made in a horse in the preparation of an antitoxine ; (*e*) by heating the cultures of the organisms, as in the case of the vaccines for black-leg in cattle ; (*f*) by drying, as in the case of the spinal cords of rabbits used in inoculation against hydrophobia (but *see* below, 5).

[1] The employment of organisms attenuated by passage through animals has been called "Jennerisation" ; the use of chemical and other methods of weakening their virulence, "Pasteurisation."

2.—The dead bodies of the bacteria may be injected instead of the living germs. This method is adopted in Wright's antityphoid vaccination and in the treatment of affections due to staphylococci, gonococci, *Bacillus coli*, and other organisms.

3.—The bacteria may be inoculated in some special way, different from that by which they normally enter the body to cause infection. Thus cholera-germs may be injected hypodermically, instead of reaching the alimentary tract by the mouth, as they do in cases of natural infection. The bacillus of black-leg (*Rauschbrand*) may be inoculated subcutaneously or intravenously for purposes of protection, its natural seat being the muscles. The tail is sometimes chosen as a site of inoculation in animals, as being colder than the rest of the body and poorly supplied with blood.

4.—An injection of antitoxic serum may be administered along with the virulent organisms, so as to neutralise part of their toxines at first, until the animal has gained for itself the power of manufacturing antagonistic bodies. This method has been used by Sobernheim in inoculation against anthrax, and by others against cattle-plague and swine-erysipelas.

5.—A very small number of the germs may be inoculated, so that the patient can overcome them naturally, whereas grave infection would be induced by a larger number of organisms. This is the principle of Hogyes' vaccination against rabies, in which a diluted virus is employed. It is not improbable that this is practically the basis of Pasteur's method in this disease, a certain number of the infective agents—those present in the external portion of the spinal cord—being killed by the desiccation, rather than all those present being reduced in virulence.

6.—The bacteria may be weakened, before injection, by treatment with the copula or immune body of their specific bactericidal serum, the complement being previously destroyed by heat. Besredka[1] has made use of this method in

[1] *Ann. de l'Inst. Pasteur*, xvi., 1902, p. 918.

vaccinating animals against the organisms of plague, cholera, and enteric fever; and Barié[1] has employed a similar method in the case of rabies. It is claimed that by this procedure the primary disagreeable effects of vaccination are avoided.

In many cases (not small-pox) there appears to be a risk in undertaking protective injections in the presence of an actual epidemic of the disease, since during the first few days after inoculation the cells of the body have formed an excess of receptors (p. 21), which, by offering more points of attachment, renders the cells more susceptible to the toxines of the disease; but the cells have not yet formed such an excess as to cause the discharge of free receptors into the serum.

The word "vaccine" is used on the analogy of the original discovery announced by Jenner, the principle being the same in the modern procedures. In diseases which have a comparatively short incubative period it is necessary to administer the vaccine before infection has occurred. In small-pox, vaccination at the time of infection may probably exercise an effect in modifying the disease, since the incubative period of vaccinia (about four days) is shorter than that of small-pox (twelve days). In hydrophobia the latent period is so long that it has been found possible to produce the immunity after the patient has been infected with the disease, but before the symptoms have appeared. This is the principle of Pasteur's antirabic inoculation, of the protective value of which there can now be no reasonable doubt (*see* p. 160).

A slightly different method, for the treatment of enteric fever, has been introduced by Jez, who employs extracts of the spleen and other organs derived from rabbits which have been inoculated with *B. typhosus*. It is supposed that bodies antagonistic to the bacilli are formed in these organs, and that they may be of use if administered to human patients suffering from the disease (*see* p. 190).

[1] *Comp. Rend. de la Soc. de Biologie*, Dec. 5, 1902.

This method is rather an instance of treatment by antitoxine than by vaccine.

PREPARATION OF BACTERIAL VACCINES

For all practical purposes these vaccines may be regarded as emulsions containing intracellular toxines in combination with bacterial protoplasm (the vitality of that protoplasm having been destroyed by heat), and the results obtained by their employment depend on the activity of these toxines and the quantity present in the vaccine. Hence a few general principles may at once be laid down with regard to their preparation. In the first place, there is a pretty general consensus of opinion that as the passage of an organism through the body of each individual host modifies to a greater or less extent its biological characters, the best results will be obtained by utilising what may be termed an "autogenous" vaccine, that is, one prepared from that particular strain of the bacterium already producing the infection of the patient, rather than a "stock" vaccine, that is, one prepared from another bacterium of the same species, but already stored in the laboratory, or isolated from another individual suffering from an apparently identical infection. The truth of this principle is borne out by the experience of numerous observers, although it is more obvious in some instances than others. For example, in an infection with *B. coli* it is futile to treat the patient with a stock vaccine; but, on the other hand, a chronic gonorrhœal arthritis will often do well with a stock preparation. In some cases the preparation of an autogenous vaccine is a matter of great difficulty, if not of impossibility, as in the chronic arthritis already instanced, owing to the difficulty of isolating the responsible organism; but it should always be attempted.

Secondly, the organism must be as virulent as possible, and to retain this character the subcultivations used in the preparation of the vaccine must not be far removed from the body of the patient. In other words, the isolation of the bacterium from the morbid material must be effected as

rapidly as possible, and in as few generations as is consistent with obtaining it in a state of purity. Hence exact and extensive knowledge of the food-requirements of the various pathogenic bacteria must be brought to bear upon the technique of the process of isolation.

Next, that particular subcultivation intended for the production of the vaccine must be cultivated under "optimum" conditions as to composition and reaction of the medium itself, temperature and atmospheric surroundings, age, and so forth; and the emulsion prepared from this subculture must be made with an indifferent fluid, and must be perfectly homogeneous and capable of sufficiently accurate standardisation to insure subsequent facility of dosage. Finally, the vitality of the bacteria contained in the emulsion must be totally destroyed, but with a minimum expenditure of heat, so that the least possible retardation may take place in the molecular arrangement of the bacterial protoplasm — that is to say, the power of vegetative multiplication must be effectually removed from all bacteria, but complete coagulation of the protoplasm should be avoided. Hence it is necessary to know accurately the thermal-death-point, in watery emulsion, of the organism under treatment, and to conduct the operation of killing the bacteria at that temperature for as short a period as will attain the desired end. Finally, after killing the bacteria, it is customary to adjust the strength of the vaccine to some convenient, although empirical, standard, and to add sufficient antiseptic to insure the continued sterility of the emulsion.

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The actual process of preparing a vaccine is briefly as follows, premising that all apparatus and reagents employed are sterile.

The organism responsible for some given infection, having been isolated from the lesion existing in the patient and identified, is planted upon a suitable medium, either in tubes to form tube-cultures, or better in a Roux or similar bottle to form "mass" cultures, and is incubated under optimum conditions for such period of time as experience shows is

calculated to yield the maximum number of vigorous living bacteria.

At the end of the cultivation-period the growth is examined visually to determine its freedom from gross contamination, and by means of stained preparations to determine its purity. The culture proving satisfactory, 5 cc. of a 0·1 per cent. saline (NaCl) solution are pipetted into the tube or bottle, and the growth emulsified as evenly as possible with the help of a glass or platinum rod. The turbid emulsion is transferred to a stout glass test-tube containing a number of glass beads: this is then placed in some form of mechanical shaker, and agitated thoroughly for about a quarter of an hour. The number of bacteria present in every cubic centimetre of the emulsion is next estimated by Wright's method. This consists in mixing equal volumes of blood from a normal individual and of the bacterial emulsion, mixing thoroughly, then spreading in a thin film on a glass slide, fixing and staining with Leishman's or Jenner's stain; and then with the help of the $\frac{1}{12}$-inch oil-immersion lens, enumerating the numbers of red cells and of bacteria respectively in some twenty-five separate "fields" of the microscope. From the numbers thus recorded an average is struck and the ratio the red blood-discs bear to the bacteria is estimated. Now, assuming that normal blood contains 5,000 millions of red cells per cubic centimetre, a simple sum in proportion gives the number of bacteria present in each cubic centimetre of the bacterial emulsion.

Having determined the numerical strength of the vaccine, a sufficient quantity of 0·1% salt-solution is added to the emulsion to reduce the numbers in each cubic centimetre to 1,000 or 100 millions, or to whatever quantity is selected as the standard, and the mixture is well shaken. The tube is then suspended for a period of thirty minutes in a water-bath running at a temperature corresponding with the thermal death-point of the bacterium employed for the vaccine (*e.g.* 59° to 60° C. for *Staphylococcus aureus*). After removal from the water-bath, loopfuls of the emulsion are sown

upon suitable media, and incubated in order to determine the sterility of the vaccine. Finally a small quantity of some antiseptic, such as 0·5% carbolic, 0·25% lysol or 0·25% trikresol, is added to the vaccine, which is then put up in small bulbs or rubber-capped bottles for use.

PHENOMENA ACCOMPANYING ACTIVE IMMUNISATION BY VACCINES

The immediate result of the introduction of a dose of vaccine into the tissues of a patient is a fall in the amount of opsonin present in the serum, owing presumably to the linking-up of some of the available opsonin to the bodies of the bacteria introduced. This is termed the "negative phase," and occupies a period lasting from a few hours to a week or ten days, or in exceptional cases a fortnight or more. Its duration is increased by a larger dose, and reduced, or even eliminated altogether, by a smaller dose. During this period, although no appreciable rise of temperature occurs (unless an excessive dose has been administered), the patient sometimes complains of not feeling well, and any local lesion that may be present is objectively worse—the discharge from a sinus increases in amount; in a cystitis there is an increase in frequency of micturition, and more pus is present in the urine: in furunculosis a fresh crop of boils may appear, and so on.

As a result of the stimulus provided by the vaccine, fresh supplies of opsonin are elaborated and discharged into the serum, and the negative phase is succeeded by a positive phase, during which the opsonin-index rises slowly or rapidly to a maximum, a subjective sense of well-being is experienced, pyrexia diminishes, often rapidly, and clinically the improvement is marked. After reaching the maximum, the index frequently oscillates slightly for a day or two, and then comes to rest, and a condition of equilibrium is established, in which the index is maintained at a higher level than it occupied before the injection, although even now not necessarily at or above the normal. This state of

equilibrium, after a period varying with different individuals, with the size of the dose, &c., declines either gradually or rapidly until it has fallen to, or below, its original position. A repetition of the dose of vaccine now causes a repetition in their entirety of the phenomena already detailed. If, however, a second dose of vaccine is administered during the negative phase induced by the first injection, a cumulative action is noted, and a second negative phase is superposed on the first: the opsonic index will then rapidly fall, perhaps with serious results to the patient. On the other hand, a second dose injected at the highest point of the positive

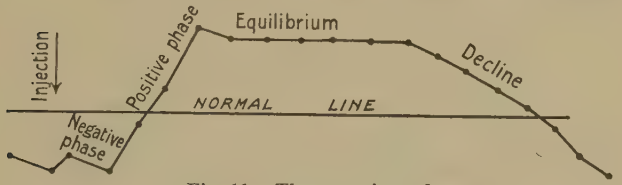

Fig. 11.—The opsonic cycle.

phase will not in most instances give rise to cumulation of positive phases (although in some infections, for example, those due to the gonococcus, *Bacillus coli*, &c., this highly desirable end can be obtained). Usually such a procedure merely results in a shorter or less marked negative phase. Practically it is found that good clinical results are obtained if the index oscillates about the normal level, provided that the greater part of the curve representing the movements of the index is above normal. Consequently, to obtain the best results by the aid of vaccines, subsequent doses should be injected towards the end of the period of equilibrium. Endeavour should be made so to adjust the dose as to obtain the shortest negative phase compatible with the production of a positive phase lasting from five to seven days.

In many acute infections, however, it is of greater importance to reduce or eliminate the negative phase which follows the injections than to lengthen the positive phase; and a dose must be administered so minute that the positive

phase appears almost immediately, and must be repeated before the transient positive phase has declined, sometimes within twelve hours.

Toxines as curative agents.—The toxines of the tubercle-bacillus have been used by Koch as a curative agent, under the name of "tuberculin." It was found that the injection of this substance caused a distinct reaction at the seat of tubercular lesions, such as lupus, and that the inflammation thus produced seemed to act beneficially on the course of the disease. In the case of the "new tuberculin" the toxic bodies of the bacteria are dissolved and injected, with the view of strengthening the patient's resistance to the disease.

THE ADMINISTRATION OF SERUM

Early administration.—The symptoms of an infective disease are due to the effects produced on the cells and tissues of the body by the toxines of its specific micro-organism, and consist in the resulting perversions of function; while the action of an antitoxic serum is to neutralise the poison circulating free in the blood and lymph, although it does not prevent the growth of the bacteria or exercise any restraining effect on them. Now, as the bacteria pour out a constant stream of toxine, and this is continually entering into combination with the side-chains (receptors) of the cells, it is most important to introduce the antidote as soon as possible, before any great amount of mischief is done. If the disease has too long a start, the antitoxine may come too late to be of any service. The great principle, therefore, in giving antitoxine of any kind is to give it at the beginning of the disease, at the earliest possible moment. In the case of diphtheria, statistics show conclusively that the power of the remedy over the disease varies directly with the promptitude of its administration, while in the case of tetanus there seems reason to doubt whether it is not already too late, in man at all events, to use the

antitoxine when the malady has declared itself. (*See* pp. 86 and 114.)

Large dose.—A second principle is to administer a large initial dose, since we do not in any case know the amount of toxine which has to be counteracted, and the supply of the latter is constantly increasing, whereas the remedy is given all at once in a single dose, and is not in any case repeated for some hours afterwards. There is also the possibility that the presence of a very large quantity of the antitoxine may tend to withdraw from the cells any poison which has already united with them. Similarly, we ought not to hesitate to repeat the dose, if it seem in the least necessary. It is better to err on the side of giving too much than too little. The danger of producing anti-antitoxine or anticomplement, previously alluded to (p. 30), does not seem to exist in practical therapeutics, though it might suggest the advisability of giving quite small doses of serum for prophylactic purposes.

Fresh antitoxine.—Thirdly, it is important that the antitoxine used should be as fresh as possible. There is evidence that the remedy tends to deteriorate in course of time. How long the different serums may retain their antitoxic powers is not yet definitely settled, and in the case of diphtherial antitoxine it seems probable that it may remain effective for at least two years. But there has been shown to be a slow process of deterioration at work in all cases, so that, in order to be on the safe side, it is well to use only quite fresh serum. If this is not to be obtained, or only after some delay, an older brand should be used rather than none at all, in preference to delaying unduly the administration of the initial dose. The same rule also applies to vaccines, which should be used fresh.

Subcutaneous injection.—Antitoxic serum is in general administered subcutaneously. It is immaterial what spot is selected for the injection; the sides of the abdomen are the favourite localities as a rule, especially the skin near the groin. The back, between the shoulders,

is equally convenient, but in the (unlikely) event of an abscess forming at the point of injection, as the result of some failure in antisepsis, the lesion would add more to the discomfort of the patient in this situation than if it were on the front of the body, where it would not interfere with the ordinary dorsal position of rest. The skin should be first washed with ether, soap and water, and then with some antiseptic, such as 1': 20 carbolic-acid lotion or with ordinary ether. The needle is passed quickly through the skin into the subcutaneous tissue, and the fluid is injected fairly slowly. The puncture is then sealed with a "scab" of collodion. In severe cases of diphtheria and in plague it has been recommended to inject the serum directly into a vein, and good results have been claimed for this method (*see* pp. 89 and 175); while in tetanus and cerebrospinal meningitis the injections should be made into the spinal canal, by lumbar puncture, after some of the cerebrospinal fluid has first been withdrawn, and also intravenously.

Administration of antitoxine by the mouth is of very doubtful efficacy (p. 18). Rectal injection is advised by Marmorek in the case of his antitubercular serum, and has been employed by some physicians in the case of diphtherial antitoxine (*see* pp. 90 and 302).

The syringe.—The serum-syringe should be of a capacity of not less than 10 cc., as this is about the largest dose of serum usually given. It should preferably have a glass piston (Fig. 12), as this can be more readily sterilised than those provided with an ordinary leather washer. The latter may, however, be made of asbestos or of indiarubber. The needle should be longer than that of a common hypodermic syringe; 2–2¼ inches is an adequate length. The bore of the needle need not be large, as the serum is perfectly fluid, and will pass readily through any hollow needle. It is unnecessary cruelty to employ the large-bored instruments often supplied, as they cause considerably more pain, and it is an advantage rather than otherwise to give the injection slowly. The serum at first causes a slight

swelling at the point where it is injected, but this soon subsides. Its diffusion may be aided by a little massage of the part, but this is quite needless in the majority of instances. If a second injection is required, it may be given at the point corresponding with the first, but on the opposite side of the body. If a series of doses is necessary, rows of punctures may be made in lines up the two sides of the abdomen.

In young infants the small size of all the parts must be borne in mind, so that the needle may not be inserted

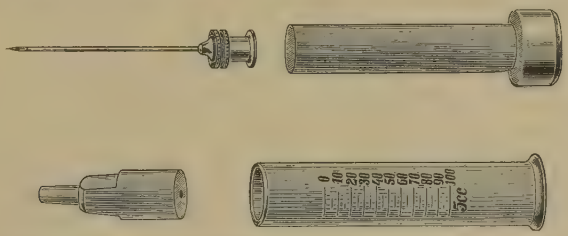

Fig. 12.—All-glass serum-syringe; readily sterilised by boiling, after separation of the component parts.[1]

unduly far. Cases are recorded in which the pleura has been punctured in the process of injection in the flanks, and gangrene of the lung has ensued with fatal result.[2] With ordinary care no fear of injury to the youngest baby need be entertained.

The syringe and needle should be boiled[3] just before use in all cases; and should be allowed to cool somewhat before the serum is drawn into them for use, to ensure that the temperature of the instrument is not such as to cause any coagulation of the albuminous fluid, whereby the needle might be blocked. After use the syringe and needle should be washed through first with cold water and then with some antiseptic to ensure sterility.

[1] Made by Messrs. Burroughs, Wellcome & Co.

[2] R. G. H. Tate, *Dublin Journ. of Med. Science*, April, 1900, p. 271

[3] In the all-glass syringe the parts should be separated before boiling, to obviate danger of cracking.

Local use of serums. — In some diseases which are characterised by distinct local lesions it has been advised to inject the serum in the neighbourhood of these, in order to procure a local effect. Thus in erysipelas good results have been obtained by injections of antistreptococcic serum into the periphery of the inflamed area, and in plague it has been recommended to make the injection into some part of the skin which is drained by the lymphatics leading to the bubo. An antibacterial serum has been used in the form of lozenges in cases of diphtheria, in addition to the use of antitoxine subcutaneously.

OCCASIONAL ILL EFFECTS OF SERUM

Effects on man of the serum of the lower animals.—It is probable that the blood-serum is not identical in composition in any two species of animals; indeed, there are variations even in that of individuals of the same species, as shown in their different degrees of immunity to diseases. We have already alluded to the poisonous effects produced in mammalian animals by injection of eel's serum, by which an actual hæmolysis is brought about. Other serums possess varying degrees of toxicity. Antitoxic and immune serums are necessarily prepared from the blood of the lower animals, and the horse is usually chosen for the purpose on account of its size, which enables a considerable quantity of blood to be drawn at a time, as well as owing to the comparatively innocuous nature of the serum of this animal in its action on man. The injection of normal horse's serum into man may, however, be followed by certain results of an unpleasant, and at times even dangerous, character. It is found that the serum of some horses manifests these qualities in larger measure than that of others. The fact that a horse has been immunised to a certain toxine or organism does not seem to have anything to do with the production of the symptoms referred to; the peculiarity resides in the serum of the animal, and is uninfluenced by the matters

used for inoculation. It is also possible that an idiosyncrasy on the part of the patient injected may be the origin of some of the ill effects noticed. The peculiar condition termed "anaphylaxis," which results after an injection of foreign serum, has already been mentioned (p. 33). Many cases of severe disturbance following the use of antitoxic serum may be attributed to this occurrence, but instances of rashes and other troubles after a first injection cannot be so explained. In such cases the hypothesis that the patient has become sensitised by the previous ingestion of horse-flesh (which on the Continent of Europe is a fairly common article of diet) has been advanced.

Nature of symptoms.—As the most frequently used serum is the diphtherial antitoxine, it is chiefly in the case of this remedy that ill effects have been observed. They consist in cutaneous eruptions of various kinds, pains with some swelling and tenderness in the joints, and occasionally rise of temperature and feeling of illness. A good deal of itching is frequently met with at the site of injection. The rashes appear, for the most part, some time after the administration of the serum (second week), and are of the type known as erythema multiforme, *i.e.* they present many different appearances, erythematous, urticarial, scarlatiniform, morbilliform, &c., but all are essentially conditions of hyperæmia and escape of serum into the tissue-spaces in varying proportions. Sometimes the hyperæmia predominates (erythema, &c.); sometimes the escape of serum (urticaria). In a very few instances more serious effects have ensued. Thus Rauschenbusch[1] records the case of a child who received a prophylactic injection of antitoxine, and who was seized with "giddiness, faintness, vomiting, and cutaneous irritation, with urticarial wheals, within a few minutes of the injection." She remained ill for some hours, but was nearly well on the following day. Actual death may occur. A few instances

[1] Quoted by Durham, art. "Antitoxins," in Quain's *Dictionary of Medicine*, 1902.

have been recorded after the use of diphtherial antitoxine (p. 98), and the present writers have seen a case in which an injection of antistreptococcic serum in a patient suffering from pernicious anæmia was quickly followed by coma and death. When, however, we consider the enormous number of injections of serum of all kinds that are given, the number of fatal cases reported—and it is probable that scarcely a single one of such fatalities escapes record from its very rarity—becomes almost infinitesimal. The risk is much less than that of the smallest surgical operation, and can be entirely neglected in the presence of any real illness or even danger of infection.

Mode of obviating ill effects.—Ill effects appear to be associated to some extent with the amount of serum used for an injection, a large dose being more likely to be followed by rashes, &c., than a small one. There is thus reason to hope that the occurrence of these symptoms will become less and less frequent with the course of time, since, as it becomes possible to prepare serums of increasing antitoxic strength, smaller doses will be required to produce the desired effects. Thus, diphtherial antitoxine has been prepared containing as much as 1,500 units to the cubic centimetre, a very minute quantity of such a potent remedy being necessary for any one injection. Attempts have also been made to separate the antitoxine from the rest of the serum by precipitating the globulin and utilising this for injection (*see* p. 81). Certain horses whose serum exhibits specially toxic qualities should not be used for the preparation of serum. Horse-serum is said to be most toxic when freshly drawn, and gradually to lose some part of its irritating qualities. Heating the serum to 60° C. also diminishes its toxicity, while previous administration of calcium chloride or lactate to the patient diminishes the risk of exudative phenomena (urticaria, &c.). Besredka [1] suggests the measurement of the toxicity of serums by estimating their power of inducing anaphylaxis (p. 33).

[1] *Ann. de l'Inst. Pasteur*, Oct., 1907.

CHAPTER III

SERUMS AND TOXINES IN THE DIAGNOSIS OF DISEASE

Agglutination-test.—Allusion has already been made to the diagnostic use of the agglutinating power which the serum derived from patients suffering from certain infective diseases exerts upon the bacteria causing the condition. The first observation of this property was made by Gruber and Durham with regard to the reaction as it affects the bacilli of enteric fever. It was afterwards found that very many kinds of micro-organisms were similarly affected by the serum of animals immunised against them. Widal first suggested the use of the reaction as a test of the existence of enteric fever, and the test is commonly known as "Widal's test" in consequence.

At first it was thought that the mere fact of a serum from a patient possessing the clumping property was conclusive evidence that the disease from which he was suffering was enteric fever; but it was soon found that the serum of many normal persons was capable of producing the same effect. That derived from typhoid patients, however, is much more strongly agglutinative than normal serum, and will produce the reaction even if considerably diluted (*e.g.* 1 : 200). The test was therefore modified, a diluted serum being employed. A one-in-ten dilution was at first considered sufficient, the method adopted being to mix on a glass slide one loopful of the serum to be tested with nine loopfuls of a fresh and vigorously-moving culture of *Bacillus typhosus*. It is necessary that the culture should be a young one, as some agglutination of the bacilli takes place

in older cultures without any addition of serum, while the bacteria move more vigorously in young cultures. It is recommended, therefore, to inoculate a broth-tube twelve to twenty-four hours before use in order to have the organisms at their best for the test. A drop of the mixed fluid—serum and culture—is brought into the field of the microscope, and the condition of the bacilli is observed. At first they can be seen moving actively about in all directions, but their movements gradually become more sluggish and finally cease, while the organisms may be seen to become aggregated into clumps or masses. A time-limit is necessary for this test, and half-an-hour is that usually taken. If within this time the bacilli have all, or nearly all, ceased to move and become massed together, then the test is said to be positive. Although the test is generally conducted at room-temperature (about 20° C.), the optimum temperature for agglutination is said by Weil [1] to be 60°–65° C.

It is now recognised that a dilution of 1 : 10 is not sufficient to exclude a number of cases in which the individual normally possesses a somewhat high agglutinative power without any infection with enteric fever. A dilution of 1 : 50 is therefore taken as the lowest dilution from which to judge of the reaction of a serum in suspected enteric fever; if such a diluted serum causes agglutination within half-an-hour, the reaction is called positive.

It is also advisable to dilute the serum itself before mixing it with the culture of bacilli, and not merely to use the latter for purposes of dilution; since the pure serum may produce some clumping on first coming into contact with the bacilli, before the whole is properly mixed, and errors may thus arise. For dilution of the serum, sterile salt-solution (0·6 per cent.) must be employed, since in the absence of salt the reaction may fail.

It was at first hoped that in Widal's reaction we possessed a certain test for the existence of enteric fever;

[1] *Prag. med. Woch.*, 1904, No. 19, p. 233.

but we now know that this is not the case. On the one hand, a certain number of undoubted cases of enteric fever fail to give the reaction at all. Fatal cases are from time to time encountered which never showed any power of agglutination, but which present *post mortem* the characteristic lesions of the disease. On the other hand, cases which are not enteric may exhibit a comparatively high agglutinative power (*see also* p. 197).

The serum of patients suffering from other diseases may possess towards the corresponding bacteria as high an agglutinative power as that found in enteric fever, or even higher degrees. Thus in Mediterranean or Malta fever it is quite usual for the serum of patients to clump the micrococcus in dilutions of 1 : 250, or even 1 : 1,000, though here, too, a dilution of 1 : 50 is recommended as a good practical working strength for diagnosis. The serum of dysenteric patients may clump Shiga's bacilli in a dilution of even 1 : 1,000 in some instances. Posselt and Sagasser[1] consider that a dilution of 1 : 50, recommended by Shiga, is here not sufficient to secure an accurate diagnosis. The serum of a guinea-pig artificially immunised against colon-bacilli may react with these organisms in a dilution of 1 : 25,000, while that of a typhoid-immunised horse may possess nearly equal strength.

The observers just quoted show that while a serum may normally possess a power of agglutinating several kinds of bacteria, the process of immunising the animal against one kind of organism will raise the agglutinative power against the others, though not in equal degree. Thus the serum of a patient suffering from dysentery may possess an agglutinative power for *B. dysenteriæ* of 1 : 300, while it may react with *B. typhosus* at 1 : 75, with *B. coli* at 1 : 30, and *V. choleræ* at 1 : 35. If examination were only made for its reaction with typhoid-bacilli, an error of diagnosis might easily result. They therefore hold that it is necessary, before accepting a reaction as positive, to test the

[1] *Op. cit.* (*see* p. 15).

agglutinating power against several organisms. It need hardly be pointed out that, if such be the case, it adds considerably to the difficulty of making the test, and thereby detracts greatly from its value for every-day use.

Another way of making use of the agglutinative reaction for diagnosis is to add a measured volume of serum to a known quantity of culture in a test-tube. If the former possesses agglutinative properties, a precipitate forms in the tube, visible to the naked eye, owing to the subsidence of the clumped bacteria to the bottom of the glass. This is known as the "sedimentation test" or the "precipitation test." It is also possible to cultivate organisms in the serum and to compare the appearances which they present with those of cultures in ordinary serum. In some cases the growth in the specific serum is characterised by clumping or by formation of chains or threads (Pfaundler's reaction).

The agglutinative power remains present in the serum long after the infection which led to its appearance has subsided. Hence, not only do convalescents from, for example, enteric fever react to Widal's test, but also persons who have suffered from the disease in previous years. How long the property remains is not known for certain. Probably it varies in different individuals, and perhaps according to the severity of the attack. In the case of enteric fever it has been proved to persist for over eight years (French and Louisson), and after dysentery it has been found to last for at least a year in some cases (Kruse). It is suggested that the duration of agglutinative power corresponds with that of immunity to the disease, but this cannot be considered proved as yet.

A drawback to the use of the test as a means of diagnosis lies in the fact that it does not appear quite at the beginning of the illness, at which time it is most needed as an aid to diagnosis. Thus, in enteric fever, it cannot be relied upon to appear before the second week of the disease; in plague it may be absent until convalescence. In dysentery

the reaction is often wanting in mild cases, according to Shiga. This author holds that the agglutinative power in any case bears a direct proportion to the severity of the infection—a contention that will not hold good in all cases, for a very mild case of Mediterranean fever exhibited a complete reaction when tested against *M. melitensis* in a dilution of 1 : 500,000.

Toxines as means of diagnosis.—It is found that in some diseases the injection into the affected animal or patient of the toxines of the bacillus causing the condition produces a febrile reaction, and use has been made of this as a means of diagnosis in the case of glanders and tuberculosis. The preparation used for the diagnosis of the former disease is known as "mallein," and is much used in veterinary practice to discover the existence of the disease in horses, in which it is often very latent. Tuberculin is similarly used on cattle to reveal the existence of tuberculosis, and has also been employed in human patients, though it has not come into general use, partly owing to the disagreeable nature of the effects produced, and to a real or supposed risk of doing harm to the sufferer ; partly from the existence of other means of diagnosis, such as physical examination, and the search for bacilli in the expectoration. It is, however, used in the percutaneous, the cutaneous, and the ophthalmic reactions (*see* pp. 269, 270).

Diagnosis by opsonic determination.—The estimation of the opsonic index may be of considerable assistance in the diagnosis of obscure conditions, for an index well above 1·2 to any given organism points in no uncertain manner to infection by that particular bacterium. Moreover, when tuberculosis, for example, is suspected and the index at the first estimation is within the normal range (*e.g.* 1·2 and 0·8), a series of observations at frequent intervals will usually reveal the characteristic movements of the patient's tuberculo-opsonin index, if the condition is actually due to the *B. tuberculosis ;* while, if it is due to some other cause, the index will remain practically steady, or, at any rate,

its curve will not show marked excursions from the normal line. Occasionally, however, it may be necessary to extend the observations of the opsonic index over a considerable period of time before the resulting curve exhibits characteristic features.

In order to obviate the delay involved by watching the natural movements of the opsonin-curve, the metabolic products of the tubercle-bacillus, in the form either of an auto-inoculation or of tuberculin (T.R), may be utilised. Thus, supposing that the case is one of suspected tuberculous disease of the knee-joint, or of the kidney, exercise of the affected joint, either by passive manipulation or by its use in active movement in the one case, or palpation and massage of the affected kidney in the other, will lead to the discharge of tubercle-bacilli or of their products into the circulation, and will produce all the phenomena described as following the subcutaneous injection of a suitable vaccine—in this case tuberculin—*i.e.* negative phase, positive phase, and so on, and will so confirm the diagnosis. If, on the other hand, the affection is not of a tuberculous nature, no appreciable movement of the tuberculo-opsonin index will take place.

If, however, it is impossible or undesirable actively to interfere with the focus of infection, a diagnostic dose of tuberculin (*e.g.* ·0002 mg. tuberculin, T.R.) may be injected subcutaneously, and its effect upon the amount of opsonin present in the blood-stream noted. For this purpose samples of blood should be taken immediately before, and also 1, 4, 12, 24, and 48 hours after the injection. All these specimens may be examined at one and the same time (using the same batch of tubercle-emulsion and of "washed" cells), and all compared with the same control "normal" serum, when estimating the opsonic content of each blood-sample. With a small dose such as this, in the case of the tuberculous lesion, the immediate transitory rise, or spurious positive phase, the negative phase and the true positive phase may be confidently anticipated in rapid sequence.

CHAPTER IV

DIPHTHERIA

Nature of diphtheria.—Diphtheria, derived from the Greek word *διφθέρα*, a skin or piece of leather, was a term originally applied to cases of sore throat characterised by the presence of "false membrane." When the condition came to be examined bacteriologically, it was found that the great majority of these cases are associated with the growth of a particular bacillus (*B. diphtheriæ*). It was therefore assumed that all cases of the disease were due to this organism, and it became the fashion to diagnose diphtheria solely on bacteriological findings. A case of sore throat in which the bacillus is found is now called "diphtheria," apart from the presence or absence of the characteristic clinical symptoms (membrane-formation), while there is a tendency to refuse to recognise cases of membranous sore throat in which pneumococci, pneumo-bacilli or streptococci are alone present and in which no diphtheria-bacilli can be detected, as instances of the disease. The practical result is to change the connotation of the term diphtheria from that of "membranous sore throat" to that of "sore throat due to *B. diphtheriæ*." A recognition of these facts will be seen to be of importance when the evidence for the value of antitoxic serum is discussed.

Causal organism.—The *Bacillus diphtheriæ* was first discovered by Klebs in the year 1883, and was cultivated by Loeffler in the following year; hence it is generally known as the Klebs-Loeffler bacillus. It belongs to a group of organisms, the exact relations between the members of which are not definitely decided. The most closely allied

form is the so-called pseudo-diphtheria bacillus, which very nearly resembles the pathogenic organism, but is not usually virulent for animals. Its relation to cases of sore throat in human beings is still undecided. Culturally it is quite distinct from the Klebs-Loeffler bacillus; moreover, it is said not to be agglutinated by the serum of animals rendered immune against the Klebs-Loeffler bacillus—strong evidence of the diversity of the two organisms. Another closely allied, if not identical, organism is the *Bacillus xerosis*, which is met with in the conjunctival sac and was at one time supposed to be causally associated with the affection of the eye known as xerosis conjunctivæ; it is also of common occurrence in the discharge of otitis media, in association with the pathogenic organism responsible for the suppuration, and is a very common saprophyte of the external genitals.

Occurrence in the body.—The Klebs-Loeffler bacillus is met with not only in cases of membranous sore throat or diphtheria, but also in chronic nasal discharges. It may also be found in a virulent condition in the throats of healthy persons.

Diphtheria-bacilli have also been found in the disease called noma or cancrum oris, and they may gain a footing on any open wound and there give rise to the formation of false membrane. In cases of membranous sore throat in which the diphtheria-bacillus is found, it may occur either in almost pure culture or mixed with other organisms, especially streptococci. These mixed cases are generally more severe, and the prognosis is worse than in simple diphtherial infection. As in the throat, so also on wounded surfaces, other bacteria, such as streptococci and the pneumo-bacillus of Friedländer, may form false membrane, so that every such formation is not diphtheritic in the strict sense of the word, *i.e.* caused by the *B. diphtheriæ*.

In cases of diphtheria the bacilli remain for the most part confined to the false membrane in the fauces; no general infection of the blood takes place as a rule, though in severe cases a condition of septicæmia may perhaps occur.

They manufacture a substance, probably of the nature of a ferment, which is absorbed by the blood-vessels and carried all over the body. This ferment, by its action on the tissues, gives rise to other poisonous materials or secondary toxines. The bodies of the bacilli themselves are not so poisonous as their soluble products; thus Kossel[1] showed that if the actual bacteria were washed free from the poison and then killed, the dead bodies had very little toxic influence when injected into animals.

Toxines of diphtheria.—The nature of the poisons manufactured by the diphtheria-bacillus was studied very early in the history of modern bacteriology, since the organisms form soluble toxines which can be readily obtained in culture-media.

Roux and Yersin[2] were the first who discovered the presence of diphtherial toxines in broth-cultures of the bacilli (1888, 1889). Solutions of the poisons may be prepared by growing the organisms in broth for periods of two to four weeks, and then either passing the fluid through a porcelain filter, so as to strain off the bacilli, or adding a germicide of some sort to it, so as to kill them. Toluol has been used for the latter purpose by Ehrlich and Wassermann.[3] The fluid is shaken well up with this substance, which separates, on standing, into a layer floating on the surface of the broth. In this condition the preparation can be kept indefinitely, as the toluol prevents any decomposition taking place. The bodies of the bacilli sink to the bottom of the flask. A special method of growing the bacteria was devised by Aronson,[4] in which they are induced to form a scum or coat on the surface of the broth; by this means they produce a much stronger toxine than when they are cultivated in the ordinary way, diffused through the fluid medium.

[1] *Centralbl. f. Bakt.*, Bd. xix., 1896.
[2] *Ann. de l'Inst. Pasteur*, 1888–9.
[3] *Zeitschr. f. Hygiene*, Bd. xix., 1893.
[4] *Berlin. klin. Woch.*, 1894, p. 426.

The effects of the poison are seen equally well whether the living bacilli or the prepared toxines are used for experimental injection. A guinea-pig which has received a dose of the organisms subcutaneously presents first at the site of injection an œdematous swelling, which is followed by enlargement of the neighbouring lymphatic glands. The animal appears to become weaker and weaker, and dies, if a moderately strong dose has been given, in the space of about four days. An examination of the body then shows the existence of œdema and hæmorrhage at the site of injection, and serous effusion into the cavities of the pleuræ, pericardium, and peritoneum. The bacilli are not found to have become generalised throughout the body. Very large doses of toxines or very virulent bacilli may produce death in twenty-four hours.

If weaker doses of poison are administered, insufficient to cause death, the most marked phenomena may be the local swelling; and if life is prolonged for as much as a fortnight, paralytic symptoms may supervene, and the guinea-pigs die of asthenia. There is reason to believe that at least three separate poisons are manufactured by the Klebs-Loeffler bacillus: one, which is the most important, causes death by a general intoxication; a second produces the local œdema at the point of inoculation, which may actually go on to necrosis of the superficial tissues; and the third is responsible for the paralysis which sometimes occurs as a sequel.

As to the exact chemical composition of the toxines little is definitely known. Roux and Yersin considered that the main poison was of the nature of a ferment; they found that the toxic substance which they succeeded in isolating did not act in the presence of acid. Sidney Martin was also led to believe that the primary poison is a ferment. He isolated from the tissues of animals dead of the disease, as well as from the culture media in which the organisms had been grown, a series of albumoses (proto-, deutero-, and hetero-albumose), as well as an organic acid. He

considered that the albumoses were formed in the body-tissues, especially in the spleen, not in the false membrane. In this latter the ferment was generated, and thence it was absorbed by the blood-vessels. Brieger and Bör[1] grew the bacilli in dialysed urine, a non-albuminous fluid, and precipitated the toxine by means of zinc chloride. The material thus prepared was non-albuminous; it was very sensitive to oxidising agents, but resistant to reducing substances. It was highly toxic to animals, and the injection of it in small quantities produced immunising substances in their serum. These observers found that the bodies of the bacteria contained a substance which was capable of causing necrosis of living tissues, and which did not give rise to antitoxine in the serum. In this respect they are at issue with Kossel,[2] who found the bodies of the bacteria only slightly toxic.

DIPHTHERIAL ANTITOXINE

Manufacture of antitoxine.—For the production of antitoxine it is necessary to prepare a toxine of the highest possible virulence. Certain strains of the bacillus appear to be specially adapted to form toxines in artificial media, taking on the peculiar form of growth already described (formation of a pellicle on the surface of the nutrient fluid), which is found to be most advantageous for this purpose. When the organisms have grown for about a fortnight on the culture-fluid, the latter is passed through a porcelain filter; the bacilli are thus removed, and the filtrate is ready for use.

The horse selected for the production of antitoxic serum is submitted to a preliminary examination with mallein and tuberculin to ensure that it is free from glanders and tuberculosis. If it fails to react to these tests, it receives an injection of a small quantity of the toxine ($\frac{1}{2}$ to 1 cc.) subcutaneously in the loose tissue at the root of the neck. The

[1] *Deut. med. Woch.*, 1896, p. 784. [2] *Loc. cit.* (p. 69).

injection is followed by considerable local reaction, causing the appearance of a large swelling, while the horse exhibits signs of fever and constitutional disturbance. It is necessary to wait till these symptoms have subsided before administering a second injection, which may be given on the opposite side of the neck. The antitoxic power of the blood rises gradually, reaching its maximum in about six months. The doses are gradually increased till as much as 1 litre of the toxine may be injected for a single dose. The febrile disturbance produced by the poison becomes less and less as the treatment continues. It appears to be a good sign that the horse should react strongly at first, as such animals seem to produce in the end a more highly antitoxic serum. Some horses fail altogether to form antitoxine; probably the receptors of their cells have not enough affinity for the toxine, and so the number killed is not sufficient to stimulate reproduction in excess.

The injection of each dose of poison is followed by an immediate fall in the antitoxic value of the serum of the horse, but this rises again in the course of a day or two to a point higher each time than that at which it previously stood. It is important not to give a fresh dose of toxine till this rise in antitoxic power has taken place; otherwise the antitoxine present may actually be diminished instead of increasing. As a rule the injections are given about once in three to seven days. A horse will not go on indefinitely producing antitoxine; its power in this direction appears to become exhausted after a time.

Standardisation of toxine and antitoxine.—As has already been pointed out, it is not possible to weigh or measure toxines and antitoxines as we do ordinary drugs, and therefore their strength can only be measured by means of physiological tests, that is to say, by determining experimentally the effects produced on living animals. For the purpose of standardising the toxines of diphtheria, guinea-pigs are the animals generally used, as it is found that they

react in a very constant manner to the poison ; those of the same weight being killed in approximately the same period of time by equal doses of a given toxine. A unit-dose of toxine is that amount of any preparation of diphtherial poison which will just suffice to kill a guinea-pig weighing 250 grammes in a period of four days. This is also known as the "minimal lethal dose (*m. l. d.*)."

A *unit of antitoxine* is the smallest quantity which, being mixed with 100 minimum lethal doses[1] of toxine and injected into a guinea-pig, prevents the appearance of any toxic symptoms.

This method of standardisation is the one inaugurated by Ehrlich. It was necessary in the first instance to establish a toxic unit, and then to calculate from this the antitoxic unit. When this had once been done, however, it became easier subsequently to calculate backwards from antitoxine to toxine, since the former is more easily preserved, not varying in strength even when kept for considerable periods of time. A standard antitoxine can now be procured from the "Serumprufungs Institut," at Frankfort-on-Maine, and is everywhere used as a standard.

Interaction of toxine and antitoxine.—From the facts just recorded it has been assumed that the relation between given specimens of toxine and antitoxine is a constant one, the same quantity of the latter being always required to neutralise exactly a certain amount of the former. This is practically true within limits. The interaction between the two substances appears, therefore, to be a simple chemical combination, similar to that which takes place between an acid and an alkali. But in the case of the substances which we are considering certain curious phenomena have been observed, showing that we are not dealing with a case of simple chemical combination. If we take a certain quantity of a simple acid and add to it the

[1] This quantity of toxine, sufficient to kill 100 guinea-pigs, and exactly neutralised by one unit of antitoxine, is called by Ehrlich the L_0 dose.

amount of the alkali which exactly neutralises it, we have a mixture corresponding with the mixture of one unit of antitoxine with 100 minimal lethal doses of toxine. If to the former mixture we add any fresh quantity of the acid, it will remain uncombined and capable of producing its normal effects (combining with more alkali, &c.). If, however, we take the mixture of toxine and antitoxine, and add to it one minimal fatal dose of toxine, we do not find that this additional toxine has still its usual effect, viz. to kill a guinea-pig of 250 grammes in four days. On the contrary, if the mixture (unit of antitoxine + 100 minimal fatal doses of toxine + 1 extra minimal fatal dose) is injected into a guinea-pig, the animal recovers from the injection, only exhibiting a certain amount of œdema at the point of injection. If still further quantities of toxine are added, it will be found that quite a large number of toxic units must be added before a point is reached at which the animal dies in four days.

This additional quantity is called by Ehrlich the L+ dose. A method of standardising antitoxine, founded on the determination of this L+ dose, has recently been introduced in place of the one described above. A standard antitoxine being available, unit doses of it are taken; varying quantities of (any) toxine are added to these, and the mixtures are injected into guinea-pigs, until the exact mixture (1 unit antitoxine and x toxine) necessary to produce death on the fourth day is discovered. This amount (x) of the toxine is then mixed with varying quantities of the antitoxine under examination, and the quantity of this latter which must be added to the above (x) amount of toxine in order that the animal may be killed in the given time, is proved to contain exactly one unit of antitoxine, its action being precisely equivalent to that of the original standard unit.

We may make the same experiment in another manner. If we take the amount of toxine which is exactly neutralised by one unit of antitoxine, viz. 100 lethal doses, add to it

$\frac{1}{200}$ of a unit of antitoxine, and inject the mixture into a guinea-pig, the animal does not die, but only suffers from some local œdema. This might, indeed, have been foretold, as there should theoretically be set free only one-half of a minimal lethal dose of poison. If, however, we proceed further in the same way, and add to the same quantity of toxine $\frac{1}{200}$ of a unit of antitoxine, we should expect death to occur on the fourth day, as one lethal dose should now be available. But again only local œdema results. Proceeding in this way, it is found that, even if $\frac{1}{250}$ of a unit is added, the mixture is still incapable of killing the animal in the stated time. When, however, the 100 lethal doses of toxine are mixed with only $\frac{1}{200}$ of a unit of antitoxine, then one minimal lethal dose is set free and the usual effect is produced. It is found at this point that for each $\frac{1}{200}$ of a unit of antitoxine that is subtracted, one lethal dose is set free. This continues till a point is reached at which we have arrived at a mixture of 100 m. l. d. of toxine with $\frac{50}{200}$ of a unit of antitoxine; this is capable of killing 100 guinea-pigs. Any further diminution of antitoxine is without effect.

Put in other words, it appears that it is possible to add to 100 lethal doses of poison as much as one-quarter of the total amount of antitoxine which will exactly neutralise them, without decreasing the available toxic capacity. If a further $\frac{1}{2}$ unit of antitoxine is added, the whole of the poison is neutralised.

The explanation of these phenomena given by Ehrlich is that the crude poison, if it may so be called—the culture-medium in which the bacteria have grown—contains several different substances, all of which have the power of combining with antitoxine. They have, however, different degrees of affinity for the latter. The body which has the greatest avidity for antitoxine is called "prototoxoid."[1]

[1] It has been suggested that toxoids consist of free "haptophore" molecules of toxine which have lost their "toxophore" element (*see* p. 22).

The main toxine, which causes the death of the guinea-pig, occupies an intermediate place in point of affinity, while a third substance, called "toxone," has the least affinity of all. This last appears to be the body which is responsible for the local œdema seen at the point of injection of diphtherial toxine.

The accompanying diagram (Fig. 13) will perhaps serve to make a little clearer what happens on gradually adding antitoxine to toxine. The tube on the left shows the

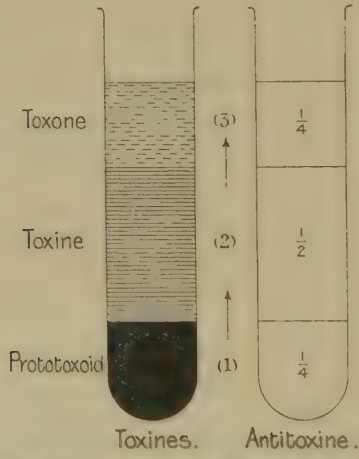

Fig. 13.—Diagram illustrating the process of saturation of diphtherial toxine with antitoxine.

relative proportions of each substance present in a specimen of crude poison. If now antitoxine be added, filling up, as it were, the tube from the bottom, it will first of all neutralise the prototoxoid, one-quarter of the whole antitoxine being thus occupied. The next two quarters will be taken up by the toxine, and the last quarter of all by the toxone. The first addition of antitoxine does not reduce the toxicity of the mixed poisons, because it merely neutralises the prototoxoid which has no poisonous properties. The second addition counteracts the most active poison, the true

toxine; while the last addition prevents the local effects which are caused by the toxone.

Again, if we take a mixture in which toxine and antitoxine are exactly neutralised (neglecting for the sake of simplicity the prototoxoid), the addition of a further unit of toxine will tend to set free an equivalent quantity of toxone, which has less affinity for the antitoxine; and on adding further quantities of toxine a fatal amount will not be reached till all the toxone has been set free and its proportion of the antitoxine annexed by the toxine.

The following illustration of the interaction of antitoxine and toxine in diphtheria, by means of an analogous process in ordinary chemistry, is given by Emery.[1]

"You remember that in estimating chlorides by titration with silver nitrate you add a little chromate of potash to the solution to be tested. The silver has a greater affinity for the chloride than for the chromate, and you get a white precipitate of silver chloride until all the soluble chlorides have been decomposed, and then you begin to get a chocolate-coloured precipitate of silver chromate. In precisely the same way, when you add antitoxine to diphtheria poison, the first portion added goes to combine with the prototoxoids, and these must be completely saturated before any toxine is neutralised."

In addition to the above facts, there are certain peculiarities about the mixture resulting from addition of antitoxine to toxine, which throw doubt on the explanation of their interaction as a simple chemical combination. Thus, a mixture of toxine and antitoxine may be made which is neutral for a mouse, but which when injected into a guinea-pig may cause toxic symptoms. Again, if the mixture be heated to 100° C., the antitoxine is destroyed and the toxine remains unneutralised. Similarly, if the mixture be passed through a porcelain filter, the toxine passes through in the filtrate, and the antitoxine remains behind; while if the same mixture be injected into an animal, the

[1] *St. Bartholomew's Hosp. Journ.*, Dec., 1902, p. 37.

toxine may be eliminated in an active condition in the urine. It seems difficult to explain these phenomena on the basis of a simple chemical combination. Some authorities have maintained that antitoxine does not act directly on the toxine, but indirectly through the medium of the living cell, which it stimulates in some way to resist the poison (Roux, Büchner). Danysz[1] considers that toxine and antitoxine may combine in different proportions to form a series of "compounds," somewhat analogous to the series of oxides of nitrogen, N_2O, N_2O_2, N_2O_3, &c.; and Bordet,[2] who also holds this view, considers that toxone is in reality a molecule of toxine incompletely saturated with antitoxine. The question is a very difficult one and cannot be decided on the data at present available. It seems probable that a definite chemical combination occurs, but that the affinity between the two substances is comparatively slight, so that the combination only takes place slowly, and is readily decomposed. Arrhenius and Madsen hold this view and consider that the results ascribed by Ehrlich to the action of toxone are really due to the presence of dissociation-products.

Von Calcar[3] states that he was able by fractional dialysis to separate the toxine from the toxone, thus proving them to be definite and distinct chemical bodies, but his methods and results have been called in question by Römer.[4]

In the case of the toxine and antitoxine of tetanus, Behring[5] considers that a third body, which he calls the "conductor," is necessary to bring about combination, this body acting in the same manner as the copula in hæmolysis. If this is proved to be the case in tetanus, it is almost certain that the interaction of other toxines and antitoxines

[1] *Ann. de l'Inst. Pasteur*, 1902, p. 345.
[2] *Ibid.*, 1903, p. 185.
[3] *Berlin. klin. Woch.*, 1905, xlii. 1368.
[4] *Beit. z. exper. Therap.*, 1905, Hft. 18–41.
[5] *Deutsch. med. Woch.*, Aug. 27, 1903.

will be found to be produced in the same manner. A somewhat similar view is propounded by Bordet and Gay[1] in the case of diphtherial toxine and antitoxine.

Some interesting experiments by Dönitz[2] throw light on the action of toxine and antitoxine within the body of a living animal. This observer found that if a dose of toxine was injected into a rabbit, it would be neutralised by the same amount of antitoxine which would neutralise it *in vitro*, provided that the latter were given within a space of nine minutes. If more than this time had elapsed, it was necessary to administer a considerably larger dose of the antitoxine; but if this larger quantity were given, it was still possible to prevent the appearance of any serious ill effects. If, however, a longer space of time than about two hours were allowed to pass after the toxine had been given and before the antitoxine was injected, no amount of the latter would suffice to avert a fatal issue. It would appear from this that we can distinguish three separate periods corresponding with distinct stages in the process of intoxication:—(1) At first the poison is circulating in the blood, and has not yet attacked the cells. (2) Later on it has entered into some sort of combination with them, but this is so loose that the presence of a large quantity of antitoxine is capable of withdrawing the toxine from them again. (3) The toxine has become so firmly fixed to the cells that no amount of the remedy is capable of undoing the combination. What constitutes the difference between the last two stages is unknown. Perhaps in the former of the two the toxine has only attached itself to the side-chains, whereas in the latter it has entered into combination with the whole body of the cell-protoplasm.

The experiments just recorded point to the necessity for the early administration of antitoxine when used as a remedy for diphtheria. It is important to give it before the poison has gained so firm a hold upon the cells that it

[1] *Ann. de l'Inst. Pasteur*, 1906, xx. 467.
[2] *Arch. Internat. de Pharmacodyn.*, Bd. 5, 1899.

can no longer be withdrawn. It is fortunate that diphtheria is a disease in which it is possible to recognise the existence of infection at a comparatively early stage, since the false membrane appears on the fauces some time before any profound intoxication of the entire system has taken place. To this fact is due, no doubt, the infinitely greater success that has attended the use of diphtherial antitoxine as compared with that prepared for tetanus. In the latter disease the existence of the infection is only recognised by the appearance of the symptoms of general intoxication. It is then, in many cases, already too late to hope for good results from the administration of antitoxine. Statistics are given later which afford incontrovertible clinical evidence of the value of early injection of diphtherial antitoxine.

Strength of antitoxic serum.—For human use it is important to possess a serum containing a large number of antitoxic units in a small volume, since it is not desirable to inject a larger quantity of the fluid than is absolutely necessary. Not only does the injection of a large dose cause a considerable local swelling, which is only slowly absorbed, but the various unpleasant effects which at times follow an injection are dependent to a great extent on the actual volume of the serum which is administered. The majority of serums on the market contain 300 to 500 units in each cubic centimetre. Hence it is not often necessary to give more than 10 cc. for a dose. Stronger specimens still can be obtained at a higher price. Diphtherial antitoxine is generally supplied in liquid form, made up with a little antiseptic as a preservative. It can also be obtained in the desiccated form. According to Chiadini[1] it appears to keep well for a period of at least eighteen months. After two years it begins to deteriorate a little, but still possesses considerable antitoxic power; after four years it is valueless. Ordinary degrees of light and heat do not affect its potency, nor does the addition of antiseptic agents.

[1] *Gaz. degli Ospedali*, 1902, No. 60.

Refined antitoxine.—Gibson[1] endeavoured to separate the antitoxine from the other constituents of the serum, by precipitation of the pseudo-globulin, which was redissolved and named "refined antitoxine." This preparation is said to retain the full antitoxic value of the original serum, and to be equally useful in the treatment of diphtheria. It also keeps well (Park and Throne). A very similar preparation has been introduced by Brieger and Krause.[2]

Value of antitoxine.—It is extremely difficult to obtain definite proof of the curative value of any drug, since the course of almost every disease is variable, and sudden improvements and relapses are liable to occur from natural causes, apart from the action of any remedy. The fluctuations are often ascribed to any drug which is being tried in the case, and there is no means by which the question, *Post hoc* or *Propter hoc?* can be decided. In the case of diphtheria the natural variations of the disease are even more marked than in many other disorders, and it is impossible to judge of the efficacy of antitoxine with any approach to accuracy in individual cases.

Dependence must therefore be placed to a great extent on collected statistics. Even here a manifest source of fallacy is introduced by the undoubted fact that infective diseases exhibit great fluctuations in virulence when viewed over considerable periods of time, the mortality from them rising and falling in accordance with obscure periodic laws which are not well understood. Hence a fall in the mortality of an infective disease may occur apart from any new remedy which has come into vogue during the period of time under consideration. In the case of diphtheria there is reason to believe that the disease has become more common in recent years, and also that the type of case seen is, on the whole, less virulent—apart from the use of antitoxine—than used to be the case. It does not seem, therefore, to

[1] *Jour. Biol. Chem.*, 1906, i. 161.
[2] *Berlin. klin. Woch.*, 1907, p. 946.

be logical to ascribe all the reduction which has undoubtedly taken place in the mortality from diphtheria to this remedy. We have to remember also that, as previously stated, there is a tendency to class as diphtheria, owing to the mere presence of *B. diphtheriæ* in the throat, cases which in earlier days would not have been considered to be suffering from this disease (*e.g.* cases of mild sore throat without any formation of membrane, which would in all probability recover without any treatment); such instances swell the number of cases of diphtheria without adding to the deaths which occur, thus reducing the rate of mortality. All these facts must be taken into account when we endeavour to form a scientific judgment as to the interpretation to be placed upon the available statistics with regard to the influence of antitoxine on the course of diphtheria. With this preliminary caution we may proceed to consider the figures actually given by different authorities.

A very instructive table is to be found in the Reports of the Metropolitan Asylums Board, giving the total number of admissions of cases suffering from each of the notifiable diseases, and the mortality which occurred in the Board's hospitals and throughout the country in each class. From it we extract the following data with regard to diphtheria.

From the table (page 83) we see that a very marked diminution has occurred in the case-mortality in the hospitals under the Metropolitan Asylums Board since the use of antitoxine became general. Reasons have already been given for thinking that not all of this apparent diminution can be rightly attributed to the new remedy, and if these statistics stood by themselves some doubt might still exist as to the value of antitoxine. But these figures are confirmed by reference to those obtainable from other parts of the country and of the world. Almost everywhere the mortality from diphtheria seems to have fallen at about the same time, and this simultaneous effect can hardly be entirely a coincidence.

TABLE[1] SHOWING ADMISSIONS FROM DIPHTHERIA TO METROPOLITAN ASYLUMS BOARD HOSPITALS FOR THE YEARS 1888–1907, WITH MORTALITY-RATE IN THESE HOSPITALS AND THROUGHOUT THE COUNTRY

Year.	Admissions.	Deaths.	Percentage Mortality in Hospital.	Annual Mortality per 1,000 Estimated Population.
1888	99[2]	46[2]	59·35[2]	0·32
1889	722	275	40·74	0·39
1890	942	316	33·55	0·33
1891	1312	397	30·63	0·32
1892	2009	583	29·35	0·46
1893	2848	865	30·42	0·76
			Average of 5 years, 33 per cent. (about).	Average of 6 years, 0·43 per cent.
1894[3]	3666	1035	29·29	0·62
1895	3635	820	22·85	0·54
1896	4508	948	21·20	0·60
1897	5673	987	17·69	0·51
1898	6566	991	15·37	0·39
1899	8676	1182	13·95	0·43
1900	7873	988	12·27	0·34
1901	7622	849	11·15	0·29
			Average of 7 years, about 16 per cent.	Average of 7 years, 0·44 per cent.
1902	6520	739	11·0	0·25
1903	5072	504	9·7	0·16
1904	4687	469	10·0	0·16
1905	4148	347	8·3	0·12
1906	5218	445	8·8	0·15
1907	5744	544	9·6	0·16
			Average of 6 years, 9·5 per cent.	Average of 6 years, 0·16 per cent.

It is noteworthy that, while the case-mortality in these hospitals has so distinctly fallen, as shown by the table, yet the mortality throughout the country generally did not decrease up to 1901. Since that date both the general and the hospital mortality has fallen notably. This decline

[1] Extracted from the Report of the Statistical Committee of the Metropolitan Asylums Board. *Annual Report*, 1907, p. 164.

[2] Number of cases too small to be of value. These figures are therefore neglected in computing averages.

[3] Treatment with antitoxine introduced during this year.

cannot, however, be altogether attributed to the use of serum, for the general mortality for the years 1871–76 was only 0·11; that for the following six years, 0·15; and that for the next five years, 0·23. There is clearly a rise and fall in the mortality apart from the use of any particular remedy.

To show that the fall in diphtheria-mortality has been general throughout the world and not confined to any one place, it may be worth while to quote statistics derived from a variety of sources. Speaking of New York, Billings[1] states that since the introduction of antitoxine a steady fall in both the number of cases and the number of deaths took place. He gives the following table, which may be compared with that on p. 83.

Mortality in New York before and after the Introduction of Antitoxine (Billings)

Year.	Cases.	Deaths.	Mortality per cent.
1891	5,364	1,970	36·7
1892	5,184	2,196	40·6
1893	7,021	2,558	36·4
1894	9,641	2,870	29·7
1895[2]	10,353	1,976	19·1
1896[3]	11,399	1,763	15·4
1897	10,896	1,590	14·6
1898	7,593	923	12·2
1899[4]	8,240	1,087	13·1

The death rate per 10,000 inhabitants previous to the advent of antitoxine was, according to Park, from 15 to 18·8. After its introduction it fell to 7, the average number of deaths in New York falling from 2,733 to 1,341 (taking the averages of fifteen years before and four years after antitoxine). In Berlin[5] the average of deaths per 100,000

[1] *New York Med. Journ.*, 1900, Feb. 17.

[2] Antitoxine introduced.

[3] Use of antitoxine became general.

[4] We have not been able to find statistics for more recent years.

[5] Cobbett, *Edinburgh Med. Journ.*, 1900, i. 521.

inhabitants in pre-antitoxine days was 90·6: it fell to 38·5 in the five succeeding years. In Paris the fall was from 62·2 to 13·3. It cannot be maintained, indeed, that all this reduction in mortality was due to antitoxine; sanitary measures probably helped to reduce the death-rate, and the virulence of the disease may have diminished; but the coincidence of a fall all over the world about the time of the introduction of antitoxine is too remarkable to be altogether accidental.

With regard to case-mortality, Rosenthal[1] collected from various sources figures showing that of 183,256 cases treated before antitoxine was introduced, the mortality amounted to 38·4 per cent. Among 132,548 cases after its use become general the mortality was only 14·6. Felix[2] states that in Roumania, before the remedy was known, the mortality of diphtherial cases was from 41 to 63 per cent.; the introduction of antitoxine has reduced the fatality among cases treated with serum to 12 per cent. Jaeger[3] states that in Mülhausen the death-rate was 52 to 55 per cent. in ordinary cases, and 65 to 68 per cent. in laryngeal cases in pre-antitoxine days; whereas it fell to 16 to 20 per cent., and 20 to 25 per cent. respectively after the use of antitoxine became general. Similarly for Vienna Siegert[4] states that:

> From 1892–4, of 4,894 cases of diphtheria, over 2,000 died; mortality nearly 50 per cent.
>
> From 1895–7, of 4,143 cases of diphtheria, only 817 died; mortality about 25 per cent.

Enough has now been said to show that the diminution in mortality is not confined to any one part of the world. Other evidence in favour of the remedy may be quoted of an even more convincing nature.

[1] *Med. Press and Circ.*, 1900, ii. 293.

[2] *Spitalul.*, 1902, No. 5 (Abstr. *Centralbl. f. inn. Med.*, 1902, p. 799).

[3] *Deutsch. Arch. f. klin. Med.*, lxxiii.

[4] *Jahrbuch f. Kinderheilk.*, Jan., 1902.

When diphtheria affects the *larynx* the cases are generally more severe than those which are confined to the fauces. Goodall[1] gives some figures as to the efficacy of antitoxine in these cases. Before the days of antitoxine, of 3,275 cases of laryngeal diphtheria, 1,008 recovered (33·8 per cent.), giving a mortality of 66·2 per cent. After the introduction of the remedy, of 3,486 cases of the same nature, 2,522 recovered (72·3 per cent.), a mortality of 27·7 per cent. Taking cases of tracheotomy, the pre-antitoxine rate of recovery was under 30 per cent.; after serum-treatment was inaugurated, the percentage of recoveries rose to 63·4 per cent. The improvement is very remarkable. Goodall concludes: "Whereas in the pre-antitoxine days, of 100 tracheotomies you could not expect to save more than 29, you can now expect to save no fewer than 53. . . . I think I am fully justified in claiming for antitoxine the great reduction in mortality among cases of laryngeal diphtheria that these figures reveal."

Park[2] records 802 laryngeal cases with a mortality of 23 per cent., and Piekema[3] 369 cases of tracheotomy or intubation with 28·2 per cent. of deaths. Both these authors ascribe the success met with to the use of antitoxine.

Still more conclusive evidence is afforded by the statistics of the comparative rates of mortality in cases in which the remedy is administered early in the disease, and in those in which a delay of some days has occurred. The reason for this has been already pointed out (p. 79).

The table on page 87 gives the results obtained by the Collective Investigation Committee of the American Pediatric Society[4] (1896).

[1] *Brit. Med. Journ.*, 1899, i. 197.

[2] *Journ. of the American Med. Assoc.*, 1900, April 14, p. 902.

[3] *Inaugural Dissertation*, Utrecht, 1900 (Abstr. *Centralbl. f. inn. Med.*, 1902, p. 799).

[4] *Journ. of the American Med. Assoc.*, 1896, ii. 27. *Cf.* Biggs, *Med. News*, 1899, July 22 and 29, pp. 97 and 137; Larkins, *Journ. Amer. Med. Assoc.*, 1899, p. 7; Park, *ibid.*, 1900, April 14, p. 902.

The records of the Brook Hospital, under the Metropolitan Asylums Board, show very similar results.[1] Thus in the years 1897–1907 (inclusive) 250 cases were treated on the first day of the disease, without a single death; of 1,513 cases treated on the second day the mortality was 4·29 per cent.; among 1,690 treated on the third day, 11·24 per cent.; among 1,338 treated on the fourth day, 16·89 per cent.; and among 1,765 cases treated on the fifth and subsequent days it was 18·58 per cent.

Thus for eleven years no death has occurred at this hospital among the cases treated on the first day of the disease.

The agreement shown among these sets of figures is very striking. There is invariably a progressive increase in the mortality as the remedy is given later and later after the onset of the attack. No stronger evidence could be found in favour of the use of antitoxine in this malady, for there is absolutely no explanation which we can adduce for

COMPARATIVE MORTALITY IN CASES OF DIPHTHERIA ACCORDING TO THE PERIOD OF THE DISEASE AT WHICH ANTITOXINE WAS ADMINISTERED

SOURCE.	FIRST DAY.			SECOND DAY.			THIRD DAY.			FOURTH DAY.			FIFTH DAY.		
	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.	Cases.	Deaths.	Per-cent. Mor.
Committee's Report ...	764	38	4·9	1,065	89	8·3	620	79	12·7	336	77	22·9	390	152	38·9
N. York Health Board Statistics	136	11	8·7	215	26	12·0	228	37	16·6	153	32	20·9	203	59	29·0
Chicago Health Board Statistics	106	0	0·0	336	5	1·5	620	18	2·7	269	38	14·1	97	33	34·0
Total	906	49	4·9	1,616	120	7·4	1,468	134	8·8	758	147	20·7	690	244	35·3

[1] *M.A.B. Rep.*, 1907, p. 165.

this steadily increasing death-rate according to the delay in the use of the remedy, other than the hypothesis of its curative power when administered sufficiently early. This accords exactly with what is theoretically to be expected of the serum and with experimental results obtained on the lower animals.

While pronouncing thus unhesitatingly in favour of the use of antitoxine, it is necessary to bear in mind that some authorities who have had good opportunities of judging of its value are still sceptical as to its usefulness. Among American writers we may mention Hermann[1] and Rupp, both of whom decline to subscribe to the general verdict in favour of the remedy. On the continent of Europe Kassowitz[3] is equally opposed to the prevailing view. He points out that, although a fall in the mortality from diphtheria was experienced in many parts of the world synchronously with the introduction of antitoxine, yet latterly the death-rate has risen again in many places in spite of its continued use. Hence the fall in the death-rate cannot be ascribed to the antitoxine. There is much truth in this argument, as has already been admitted; and if we had only this means of judging of the value of antitoxine, it would be necessary to return a verdict of "not proven." But the evidence available as to the progressively greater mortality in cases of diphtheria, according as they are left for increasing periods of time without antitoxine, appears to constitute irrefragable proof of the value of the remedy; and the expression of doubt as to its efficacy becomes increasingly rare in medical writings.

Mode of administration of antitoxine.—As a rule diphtherial antitoxine is administered *subcutaneously*, the injections being given in the flank or groin. The site of injection is immaterial. If the child is restless, it may be advisable to interpose a piece of rubber tubing between the

[1] *Med. Record*, Jan. 20, 1900.
[2] *New York Med. Journ.*, Jan. 27, 1900.
[3] *Therapeut. Monatsh.*, 1902, pp. 223, 499.

needle and the syringe in order to lessen the risk of breaking the former in the wound. Antiseptic precautions must be observed, the skin being cleaned up with soap and water and then with lysol or some similar antiseptic.

In severe cases it has been recommended to give the remedy *intravenously*, in order that it may be more quickly absorbed and so manifest its effects more speedily.

In order to avoid any risk of injecting air into the vein it is necessary to see that the needle is full to the end with the serum before it is passed into the vein. There is in reality no danger to be apprehended, if only a bubble or two of air enter a vein; and in veins at a distance from the heart there is not a sufficient negative pressure to suck air in, apart from any injected. Still, care should be taken in this respect, a finger being kept on the vein on the central (cardiac) side of the point incised, to prevent any possibility of mishap.

The serum should be warmed to body-temperature before it is used intravenously; and if it exhibit any undue opacity or deposit, it should be strained through sterilised muslin before it is drawn into the syringe for injection.

For the purpose of intravenous injection a general anæsthetic is sometimes recommended in the case of a child. For an adult cocaine-anæthesia would probably be sufficient, although we have never found either a local or a general anæsthetic necessary. Any vein may be selected, the median basilic at the bend of the elbow being that most usually chosen. The vein should be compressed above the bend of the elbow by the thumb of an assistant and the needle introduced through the skin into the subcutaneous tissue at a slight angle with the direction of the vessel. Then the direction of the point is slightly altered, and it is thrust through the wall of the distended vein. As soon as blood appears in the barrel of the syringe, the piston should be slowly and steadily pushed down, and the dose of serum injected into the vein. As the opening into the vein is small, pressure alone suffices to stop hæmorrhage.

It would seem advisable to make use of the intravenous method of injection in cases in which the symptoms are severe, and especially in those in which the use of the serum has been unduly postponed. Good results are reported by Cairns [1] from this mode of procedure, very large doses of antitoxine being used in some cases (*see* below).

The antitoxine has also been given by some physicians by the *rectum* and by the *mouth*. Dominicis [2] was one of the first to adopt this procedure and reported five successful cases. Parkinson [3] states that rectal administration has been carried out at the London Temperance Hospital, and that the results obtained have been very satisfactory. He recommends this method as being free from some of the disadvantages of subcutaneous injection, such as local abscess-formation, which sometimes follow neglect of aseptic precautions. Paton,[4] who advises the use of diphtherial antitoxine in septic conditions not due to the *B. diphtheriæ*, administers the remedy by the mouth, stating that it is not affected by digestion; and Zahorsky [5] advises the administration of antitoxine in milk to children when a prophylactic dose is needed. Pilcher [6] also commends the oral method of administration, and Chantemesse [7] has given antitoxine by the rectum with satisfactory results. On the other hand, the results of experiments, from which mild infections and the possibility of coincidence have been carefully eliminated, show that this procedure (*see* p. 18) is ineffective and unscientific; and since there is no doubt that antitoxine acts well when administered by hypodermic injection, while the drawbacks to this method are very insignificant, it seems wiser to use this method (or the intravenous) for the treatment of the disease.

[1] *Lancet*, 1902, ii., Dec. 20.
[2] *Gaz. degli Ospedali*, July 19, 1896.
[3] *Brit. Med. Journ.*, 1903, June 20.
[4] *Australas. Med. Gazette*, 1902, Feb. 20.
[5] *Archives of Pediatrics*, March, 1899.
[6] *Brit. Med. Journ.*, 1904, ii. 1751.
[7] *Sem. Méd.*, July, 1896.

Prophylactic use of antitoxine.—It has been ascertained beyond reasonable doubt that the administration of a comparatively small dose of antitoxine will produce immunity to the disease for a certain period of time. Ronald French made a series of observations upon nurses to whom 3,000 units of antitoxine—an excessive prophylactic dose—were administered before they went on duty in diphtheria-wards, and found that the diphtherial opsonin-index at first rose rapidly, reached a maximum in from 4 to 11 days, then fell to a point below the normal, and returned to the normal in about 3 weeks after the injection. Netter[1] originally showed that the protection begins about the end of the first day (24 hours) after the injection, and lasts for about 3 weeks. The protective substance is probably excreted in the urine, &c., or possibly anti-antitoxines may be formed. There seems no reason to hold that the length of the immunity is at all proportional to the dose administered. As a result of Netter's communication and the discussion which followed it, the Academy of Medicine (Paris) passed resolutions[2] to the following effect:—

1. Preventive injections of 1,000, or at most 2,000 units of diphtherial antitoxine produce immunity to the disease. This protection is transitory in character.
2. Such preventive injections are specially to be recommended in the members of families in which cases have occurred, in order to immunise the other children.
3. They are also called for in schools, crèches, hospitals, &c., where children are collected together so that infection is easily spread from one to another.
4. The injections of antitoxine are useful in patients suffering from scarlet fever and measles, in which affections diphtheria is a frequent complication.

[1] *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902.
[2] *Brit. Med. Journ.*, 1902, i. 997.

5. The prophylactic use of antitoxine does not preclude the carrying out of ordinary measures of disinfection and isolation.

In the presence of an epidemic of diphtheria, among 491 children exposed to infection, who did not receive protective injections, 87 contracted the disease; while of 502 children, who had been similarly exposed and were given protective injections, only 13 became infected. Of these, 7 developed the disease within 24 hours, and 6 more than a month after the injections. Since the immunity does not begin for 24 hours and passes off in approximately 3 weeks, these figures strongly support the prophylactic use of the remedy.[1] It might be well, owing to the transient nature of the immunity obtained, to repeat the dose if the epidemic continued, so as to renew the protection. Even apart from re-infection, virulent bacilli have been known to remain present in the throats of children for long periods of time.

American writers are strongly in favour of the preventive use of antitoxine. Biggs[2] states that out of 3,109 cases in which this was given, only 9 children acquired diphtheria; and Park[3] records 6,506 cases of immunisation, among which 28 developed the disease within 24 hours, before the protection was effective, while only 27 were attacked after this limit of time, of whom one died of scarlet fever. Billings[4] attributed a rise which had occurred in the diphtheria-rate to the neglect of the prophylactic use of the remedy.

On the other hand, Violi[5] considers that antitoxine is not a sure preventive of diphtheria, and advises that it should only be given when there is a certainty that a child has been exposed to infection. The difficulty which exists in judging of the value of any prophylactic has

[1] Netter, *loc. cit.*
[2] Quoted by Billings, *op. cit.*
[3] *Journ. of the Amer. Med. Assoc.*, 1900, i. 902.
[4] *New. York. Med. Journ.*, 1900, lxxi. 234.
[5] *Pediatria*, June, 1900.

been emphasised by Peters,[1] who records instances of failure to protect persons in whose fauces the *B. diphtheriæ* had actually effected a lodgment. Netter[2] admits that such injections need not be given if the children can be kept under observation and apart from others whom they might infect.

It cannot be denied that there is a real, though exceedingly small, risk in the administration of antitoxine. Nevertheless, as 500 units is an efficient prophylactic dose, and can be obtained in half a cubic centimetre of serum, even in private practice among well-to-do patients it seems advisable to give preventive injections as a routine procedure. In the case of institutions, such as schools and hospitals, the wisdom of this course is even clearer; here there is a very great liability for the infection to spread from one patient to another, and this precautionary measure seems imperatively necessary. It is well to remember the frequency with which epidemics of diphtheria are kept alive by means of children who are the subjects of chronic nasal discharges. This condition seems to have little effect on the health of the child itself, but the virulent bacilli contained in the nasal secretion are capable of infecting others. Hence, children who have been brought into contact with such "carriers" must be looked upon as having been exposed to infection and treated accordingly. In the management of epidemics of diphtheria, particularly those which may be designated "school epidemics," the most expeditious, economical and efficient method is undoubtedly that which includes the examination of swabbings from the entire community, segregation of all carriers, and the prophylactic injection of antitoxine into all contacts.

Dose of antitoxine.—The tendency at the present time seems to be in the direction of giving large doses of antitoxine. For *prophylactic* use 150 units were at first generally employed in America, but this dose was found

[1] *Brit. Med. Journ.*, 1907, ii. 865.
[2] *Loc. cit.*

to be too small, and the Health authorities[1] there now recommend the use of not less than 300 units. Jump[2] recommends 250 units for children under 2 years, 500 for older children and adults. The Paris Academy, whose resolutions were alluded to on pages 91–92, speak of 1,000 to 2,000 units as a protective dose. There can be little doubt that these last are unnecessarily large. Perhaps, on the ground of American experience, we may consider that 300 to 500 units is the proper dose—no distinction being made between children and adults; or if a distinction is made it should be in the direction of an increased dose for the infant. It seems advisable to repeat the dose, if liability to infection continues, or if virulent bacilli should still be found in the child's throat.

For purposes of *treatment* much larger amounts are required. Villy[3] advises that in cases of moderate severity 2,000 units should be given at once and repeated in 12 hours, if necessary. In severe cases, 8,000 to 12,000 units may be the first dose, followed by 2,000 to 8,000 units every 12 hours. McCollom[4] gave an initial dose of 8,000 units in a severe and apparently hopeless case, following this up with 4,000 units some hours later, and repeating the dose every 4 to 6 hours, till 92,000 units in all had been administered. The patient recovered completely. Satterthwaite[5] states that the initial dose for an infant under 1 year is now established at 2,000 units, for one over 1 year, 3,000; and for an adult, 4,000 to 6,000 units. Cairns[6] puts the doses for subcutaneous injection at from 4,000 to 20,000 units; while intravenously he administers from 20,000 to 35,000 units. He holds that in severe cases an initial dose of 20,000 units is not excessive. Welden[7] finds that the best results are obtained by administering 2,000

[1] Billings, *New York Med. Journ.*, 1900, lxxi. 234.

[2] *Philad. Med. Journal*, Jan. 11, 1902.

[3] *Med. Chronicle*, 1900, ii. 241. [4] Quoted by Satterthwaite.

[5] *Med. News*, May 16, 1903, p. 936. [6] *Lancet*, Dec. 20, 1902.

[7] *New York Med. Journ. and Philadelphia Med. Journ.*, Nov. 14, 1903, p. 927.

units every 3 hours until the severity of the symptoms diminishes.

On the other hand, some authorities recommend the use of small doses for treatment. Thus Musser [1] advises that children from 6 to 8 years old should receive only 500 units, while those over 8 should have 1,000, repeated if necessary in 8 to 12 hours. Geffrier and Rozet [2] also recommend that small doses should be used; and these writers do not advise the use of prophylactic injections, on account of their occasional bad effects.

Dose of Diphtherial Antitoxic Serum (in Units) Recommended by Various Writers

Author.	Mild Case.	Average Case.	Severe Case.	Prophylaxis.
Cohn [3]	1,000	—	1,500–2,000	—
Baginsky [4]	1,500	4,000	5,000	—
Bishop [5]	2,000 (adult) 4,000 (child)	—	12,000 (max.)	—
Rudolph [6]	—	3,000 repeatd.	—	300–500
Berg [7]	—	3,000 repeatd.	—	—
Pexa [8]	—	3,000	—	—
Fischer [9]	2,000	5,000	10,000	—
Souza [10]	—	7,000	—	—
McCollom [11]	4,000	8,000 repeatd	12,000 repd.	—
Rolleston [12]	3,000–12,000	12,000–18,000	18,000–24,000 (repeated)	—
Ibrahim [13]	—	—	—	250–500
Wesener [14]	—	—	—	300–400
Aaser [15]	—	—	—	300–400

[1] *University Med. Magazine* (Philadelphia), March, 1900.

[2] *Arch. de Méd. des Enfants*, Feb., 1900.

[3] *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, Bd. xiii., Hft. 4 and 5.

[4] *Berlin. klin. Woch.*, 1908, p. 1257.

[5] *Brit. Med. Journ.*, 1907, ii. 1528.

[6] *Brit. Med. Journ.*, May 9, 1903. [7] *Med. Record*, Nov. 26, 1904.

[8] Abstr. *Centralbl. f. inn. Med.*, 1905, p. 975.

[9] *Med. Record*, 1904, lxvi. 875.

[10] *Gaz. dos Hospitales do Porto*, Aug., 1907.

[11] *Med. Record, loc. cit.* [12] *Practitioner*, 1904, lxxiii. 615 *et seq.*

[13] *Deut. med. Woch.*, Mar. 16, 1905.

[14] *Münch. med. Woch.*, 1905, No. 12.

[15] *Berlin. klin. Woch.*, Sept., 1905.

Our own opinion inclines to the administration of an initial subcutaneous dose of 2,000 units for an infant, and 4,000 for an adult, repeated 12 and 24 hours later, if necessary; this may be accompanied, if the case is a very severe one or is first seen at or about the fifth day, by an intravenous injection of a similar amount.

The opinion has been expressed that the use of antitoxine after the fifth day of the disease is ineffectual and should be discontinued.[1] Its use in late stages has even been looked upon as actively harmful. Such views are vigorously combated by Rolleston,[2] and are contrary to the accepted theory of the nature of the disease. We believe that in any severe case of diphtheria antitoxine should be administered as soon as the case comes under treatment. In cases of relapse serum should again be injected in spite of the probability that severe symptoms of "serum-disease" may ensue. It is well, however, to bear in mind the condition named by Rolleston "angina redux" (the *angine de retour* of French writers), a form of simple tonsillitis which does not call for specific treatment.

Antitoxine and post-diphtheritic paralysis.—There is reason to believe that since the introduction of antitoxine the percentage of cases which suffer from paralysis after diphtheria has definitely increased. This effect was at first attributed to the remedy, and some prejudice against the use of it was thereby excited. Since, however, as we have just endeavoured to prove, it saves the lives of many patients who would otherwise have died, and these severe cases are those which are most likely to exhibit paralysis later on, there inevitably arises an increase in the percentage of cases of paralysis; it is in reality a testimony to the value of the antitoxine, and not a drawback to its use.

With regard to the incidence of paralysis after diphtherial intoxication, Ransom[3] comes to the following conclusions as the result of experimental researches:—

[1] Bolton, *Lancet*, 1907, i. 870. [2] *Ibid.*, Ap. 20, p. 1112.
[3] *Journ. of Pathology and Bacteriology*, 1900, p. 397.

"1. Paralysis may certainly be expected after intoxication with not less than one-quarter of a minimal fatal dose. With doses between one quarter and one-eighth of this amount paralyses occur, but are not constant, and below one-eighth no paralysis was noticed.

"2. The larger the dose of toxine the severer will be the paralysis, if the animal survives long enough.

"3. Neutralised mixtures of toxine and antitoxine containing only about one lethal dose or less do not appear to cause paralysis.

"4. Antitoxine, given 15 to 22 hours after intoxication with doses of toxine not greater than the lethal dose, exercises in large doses a mollifying influence on the subsequent paralysis. . . . Small doses of antitoxine have no evident effect in diminishing the paralysis.

"5. Transferring the results to practice among human beings, we may expect liberal doses of antitoxine given early in the illness to influence favourably the subsequent paralysis; and this favourable influence is likely to manifest itself, not so much in the local paralyses (soft palate, &c.), as in such fatal symptoms as failure of the heart. Severe cases are, however, likely to be followed by some paralysis in spite of even large doses of antitoxine."

It would appear then that so far from having any part in the production of paralysis, antitoxine has some power of restraining it. These results are in accordance with those of other writers.[1] Thus, Rosenau and Anderson found that in guinea-pigs one unit of antitoxine given before or at the time of infection entirely prevented paralysis; that if it were given within 24 hours it modified the subsequent paralysis; but that at later periods it had no influence. Comby[2] on the other hand advises the use of

[1] Malynicz ("Ueber d. Häufigkeit der postdiphterischen Lähmungen vor u. nach Serum-behandlung," Zurich, 1908) gives statistics to show that not only is paralysis more common after serum-treatment, but that cardiac paralysis is specially common and fatal. We do not think that this is the usual experience.

[2] *Lancet*, 1906, ii. 54, 243.

antitoxine in the treatment of paralysis which has actually occurred.

PERCENTAGE INCIDENCE OF PARALYSIS ACCORDING TO DAY OF TREATMENT WITH ANTITOXINE[1]

Approximate Date of Treatment.	Petit.	Monti.	Reichsfald.	Rolleston.
1st day	—	—	—	5·5
2nd ,,	6·25	—	25	15·09
3rd ,,	19	8	33	18·07
4th ,,	24·70	12	—	28·07
5th ,,	—	33·3	50	35
6th ,,	—	50	—	34
7th ,,	38·70	66·2	—	19

Ill effects of antitoxine.—The manner in which the injection of the serum of any species of animal into an individual of another species is liable to be followed by toxic symptoms, has already been considered (p. 58).

It cannot be denied that in a certain number of instances the injection of diphtherial antitoxine has been followed by *death*, directly attributable to the action of the serum. A melancholy instance was afforded by the sudden death of Professor Langerhans' infant son soon after a prophylactic dose of the serum, but this has been attributed to the existence in the child of the status lymphaticus, which predisposes to death from trivial causes. A similar case of sudden death is recorded by Boone[2] in a boy aged ten years, who after an injection of 1,000 units of antitoxine suddenly sat up, clutched at his throat, became deeply cyanosed and died. No cause of death was discovered at the necropsy. Most of the fatal cases recorded have been of the same sudden character. A case in which death took place at a later period, and was due to the same vascular disturbance which gives rise to the rashes often seen after injections of anti-

[1] Rosenau and Anderson, Pub. Health and Mar. Hosp. Service, U.S.A. *Bull. Hyg. Lab.*, No. 38, 1907.

[2] *Journ. Amer. Med. Assoc.* 1908, l. 453.

toxine, is recorded by Gerlach.[1] In this instance an erythematous eruption appeared on the eleventh day after the injection; on the twelfth day there were clonic spasms, without the presence of albumin in the urine or any symptoms of uræmia. At the necropsy there was found an extra-dural meningeal hæmorrhage, which was attributed to a leakage from the vessels, analogous to the escape of serum, and sometimes of blood, seen in erythema multiforme. Gerlach alludes to another case in which *cerebral symptoms* came on after an injection, but which did not end fatally.

Holladay records a case [2] in which a man of twenty-six received an injection of only 500 units. This was followed by tingling in the arm, where the dose had been administered, cyanosis, constriction at the chest, and collapse; recovery subsequently ensued.

Saward [3] records two cases in which sudden *syncope* occurred after injections of antitoxine. It must be remembered, however, that the toxine of diphtheria acts on the cardiac muscle, producing a tendency to syncopal attacks; it is therefore very doubtful whether these cases were really to be attributed to the antitoxine, since they might equally well have been caused by the disease itself.

An **exudative tonsillitis** may occur as a serum-phenomenon (*angina redux*, Rolleston), and may give rise to fears of a relapse; there are, however, no diphtheria-bacilli in the exudate, and this is not a distinct membrane, but a soft deposit. It is important to distinguish this condition, which only occurs with other manifestations of serum-disease, from a true relapse, as it would obviously be improper to inject more antitoxine.

Swelling of the cervical glands is another rare effect of serum-treatment. Bolton [4] alludes to the occurrence of

[1] *Therapeut. Monatsh.*, April, 1903, p. 198.

[2] *Virginia Med. Semi-Monthly* (quoted *Indian Lancet*, March 23, 1903, p. 481).

[3] *Brit. Med. Journ.*, 1902, i. 1025.

[4] *Lancet*, 1899, i. 891.

abscesses at the site of injection, and even of *necrosis* of the overlying skin in one case. Such incidents are however, probably due to some failure of antiseptic precautions.

The most frequent ill effects brought about by injection of serum are **cutaneous eruptions** [1] of various kinds. Park [2] observed them in 3 per cent. of his cases, Stanley [3] in over 25 per cent., and Villy [4] in 35·2 per cent. The rashes are generally classified as (1) erythematous, (2) scarlatiniform, (3) morbilliform, and (4) urticarial. They are all manifestations of a condition which may be termed erythema multiforme, consisting in a tendency to vascular dilatation of different degrees and distribution, and escape of serum into the tissues (urticaria). It appears that different brands of serum tend to produce different types of eruption.

1. *Erythema*, or simple localised hyperæmia, is generally regarded as the commonest form of rash. It may take the shape of a slight blush, either at the point of injection or elsewhere, or may consist of slightly raised circinate patches, which may coalesce to form gyrate patterns. Favourite seats of this variety are the extensor surface of the limbs. Out of 112 cases of rash noted by Stanley, 58 were simple erythema. Its average date of appearance after the injection was the twelfth day, varying from the fourth to the twenty-ninth. It may be combined with urticaria (15 cases).

2. The *scarlatiniform* rash is a more pronounced form of the erythematous; it is more intense and becomes widely generalised. Stanley noted this variety in six cases out of 112. It is often followed by desquamation. Leiner [5]

[1] Galitsis, *Thèse de Paris*, quoted in *Journ. de Méd. et Chir. Pratiques*, Sept. 25, 1903, p. 692, states that some eruptions occurring in diphtheria are due to infection with a special organism, *Diplococcus hemipholus*, and are wrongly attributed to the serum.

[2] *Brit. Med. Journ.*, 1902, i. 386.

[3] *Op. cit.*

[4] *Op. cit.*

[5] *Wien. klin. Woch.*, 1902, No. 43.

states that this form of eruption tends to come out within the first five days after injection—an earlier period than that of most of the other varieties; that it starts from the point of injection; that it is followed by peeling; that it is contagious, and that it seems to protect against infection with scarlatina. The infection is difficult to eradicate from a ward in which cases have occurred. He concludes that it is true scarlatina, and compares it with the surgical variety of this disease. It is very possible that the cases observed by Leiner were of this nature, and there is no doubt that the diagnosis between a serum-rash and an attack of true scarlet fever must be difficult; but there is no reason to suppose that all cases of scarlatiniform rash after the administration of antitoxine are of this character. The co-existence of diphtheria and scarlatina is, however, not very infrequent.

3. *Morbilliform eruptions* formed less than 3 per cent. of Stanley's cases. They may be accompanied by swelling of the face, conjunctivitis, and lachrymation, so as exactly to resemble measles. Even pyrexia may occur. Distinguishing points from true measles are that the rash comes out first on the limbs, instead of on the face and behind the ears; that gyrate patterns are met with; and that there is no accompanying bronchitis (Villy).

4. *Urticarial eruptions* are common. They occurred in 30 of Stanley's 112 cases. They are met with at an earlier period after injection than the erythemata—from the fourth to the nineteenth day (average, ninth, Stanley). The urticaria may be quite transient, or may last several days. The itching is sometimes very severe. A local urticaria round the site of injection may be seen.

Pains in the joints, often accompanied by swelling, erythema, and pyrexia, are another inconvenience which may arise after injections of serum. They are not usually severe, but Taillens[1] records two cases in which they were accompanied by high fever and a rash; in one of his cases

[1] *Revue Méd. de la Suisse Romande*, July 20, 1903, p. 463.

the pains were so intense that the child could not move at all, or bear the weight of the bed-clothes to rest on her. Villy noted joint-pains in 6·5 per cent. of his cases.

Successive crops of cutaneous eruptions or attacks of joint-pains often correspond with successive administrations of serum.

Albuminuria may follow the use of antitoxine, but is not of any serious import. Actual *nephritis* is said to be rather diminished than increased by the use of this remedy, and certainly existing albuminuria does not contra-indicate the use of antitoxine. *Suppression of urine* is also more rarely seen in these days (Villy). *A rise of temperature* as a result of the antitoxine was observed by this writer in 19·8 per cent. of his cases.

An interesting case illustrating the effects of *idiosyncrasy* in relation to antitoxic serum is reported by Reckles.[1] He administered an injection of 4,000 units of antitoxine to a woman who was suffering from faucial diphtheria. A few days later, when she appeared to be convalescent, a severe urticaria developed, and shortly afterwards she was seized with a sudden attack of præcordial pain and dyspnœa, with marked lividity of the face. In the next few days nine successive attacks of this nature were experienced, but in the end the patient recovered satisfactorily. It was subsequently learnt that two years previously she had received an injection of antitoxine for diphtheria, and that she had on that occasion also suffered from attacks of dyspnœa and cyanosis.

Borchman[2] states that antitoxic serum produces less ill effects if it is warmed to 58° C. before it is injected. This has only a slight effect in diminishing its potency. He quotes his experience in 578 cases; in 193 of these the remedy was given in the ordinary way, cold, and among these there were 22·7 per cent. of rashes. On the other

[1] *Quarterly Med. Journ.*, Feb., 1903.

[2] *Dietskaya Meditsina*, 1900, v., No. 3. (Abstr. in *Pediatrics*, 1900, vol. x., p. 316.)

hand, among 385 patients who received the warm serum, only 16·3 per cent. developed these troubles. Courmont[1] reports that no rashes or anaphylaxis follow the intravenous injection of serum. Rolleston regards the occurrence of serum-rashes as of good prognostic import.

Antitoxine in conjunctival diphtheria.—Antitoxine has proved of the greatest service in diphtherial infection of the conjunctiva. Emmett Holt[2] states that without it total destruction of the eye generally results, whereas with its aid good results may be obtained. He administers 2,400 units as a dose. Stevenson[3] also bears testimony to the value of the remedy, as the result of a study of 43 cases; he advises the use, along with the antitoxine, of a lotion of perchloride of mercury (1 : 5,000). The diphtherial nature of the affection must be established by bacteriological examination.

Post-scarlatinal ear-disease.—On the other hand, curiously enough, in cases of middle-ear disease following scarlatina, in which diphtheria-bacilli appear to be the pathogenic organisms, antitoxine is said to act neither as a prophylactic nor as a curative agent, although the patients seem to obtain immunity to faucial infection by the bacilli.[4]

Cancrum oris.—Some cases of noma, or cancrum oris, are due to the *B. diphtheriæ* and should be treated with antitoxine. Telford[5] records a successful case.

Diphtheria as a complication of other diseases.—Diphtheria is liable to occur as a complication of scarlet fever and of measles. In the former malady the supervention of diphtheria constitutes a very grave condition, the mortality being very high. In measles, also, infection with diphtheria is very likely to occur. It has, therefore, been

[1] *Soc. Méd. des Hôp.*, 1905, p. 504.

[2] *Pediatrics*, May, 1902.

[3] *Brit. Med. Journ.*, March 22, 1902. *Cf.* Strzeminski, *Recueil d'Ophtalmol.*, Oct., 1905.

[4] Duncan Forbes, *Journal of Pathology and Bacteriology*, 1903.

[5] *Med. Chron.*, 1906, p. 221.

suggested that prophylactic injections of antitoxine should be given as routine treatment in both these diseases. If many cases of such super-infection have occurred in a scarlet-fever ward, then such treatment would certainly be advisable. Heubner [1] states that a larger dose of the serum is needed to produce immunity in cases of measles than under other conditions (twice the amount), and that the duration of the protection is also shorter. Richardière [2] records that in the first four months of the year 1901 two to four cases of diphtheria used to occur each month in the measles-ward of a hospital. After this time all the children as they came into the ward were injected with antitoxine, and no more cases of diphtheria occurred.

Nasal diphtheria.[3] —The ordinary acute faucial diphtheria may spread to the nose, and such cases show a very high rate of mortality. Large doses of antitoxine are called for by way of treatment. There is also a chronic nasal inflammation associated with the formation of membrane (membranous rhinitis), which affects the nose, and in which virulent diphtheria-bacilli are found. The patients do not suffer from the toxic symptoms characteristic of diphtheria, nor do they exhibit as a rule any distinct signs of ill health; but the discharge from the nose is capable of conveying the infection to others. Indeed, it is probable that many epidemics of diphtheria, of which the origin has been difficult to understand, might be traced to such chronic nasal disease, which may pass as an ordinary "cold in the head." Antitoxine often acts most successfully in these cases.

[1] Quoted by Netter, *Bull. de l'Acad. de Méd.*, Paris, March 18, 1902.

[2] *Ibid.*

[3] In this connection we may note that Myguid (*Journal of Laryngology*, Aug., 1898) made use of injections of diphtherial antitoxine in ten cases of *ozæna*. He found that the injections produced congestion of the mucosa of the nose and subsequent formation of crusts, while the fœtid odour disappeared. It does not seem to be suggested that the *B. diphtheriæ* is the cause of ozæna, the nature of which is not well understood. Myguid's experience with antitoxine is analogous

Diphtherial antitoxine in other diseases.—Various writers have detailed their experience with antitoxine in diseases other than diphtheria. Thus Talamon and Gay have used it in pneumonia; Paton recommends it for all septic conditions, and thinks it has a definite influence on inflammatory tissue; Schapiro and Tsvietaieff, and Mastri and Tomaselli have tried it in erysipelas; and Konarzsherski believes that it will cure whooping-cough. Del Monaco believes it to be efficacious in aphthous stomatitis, Sizemski in ozæna, and Gillett used it with benefit in a case of asthma—not a microbial disease. It is difficult to take these results seriously, since the most definitely established fact at present with regard to serums is their specific nature, *i.e.* the power possessed by each of counteracting the poisons or killing the bacteria of that disease alone for which it is manufactured. It has, indeed, been suggested that diphtherial antitoxine may act as a stimulant to the production of leucocytes generally, and so may be useful in other diseases in which leucocytosis is beneficial, much in the same way as cinnamic acid is said to act in tuberculosis. Thus, R. French has observed a gradual but steady rise in the tuberculo-opsonin index following the administration of 3,000 units in a case of tuberculous disease of the hip. Bradshaw[1] records a distinct rise in the tuberculo-opsonin index shortly after an injection of anti-diphtherial serum, followed, however, by a marked fall —from one to three months later. In spite of these observations, the probabilities point at present rather to the supposed benefits of antitoxine in other diseases being due to fallacies of observation, which are difficult to avoid in studying the action of any remedy.

to that of other observers in the different maladies alluded to in the text, in which it is difficult to believe that the good results seen were in reality due to the antitoxine. Our present knowledge of the action of serums is, however, too small to enable us to pronounce with any degree of confidence as to what they can or cannot do.

[1] *Lancet*, 1906, i. 1387.

ANTIBACTERIAL SERUM

Antitoxic serum, as already explained, does not tend to kill the bacilli which cause diphtheria. They will grow readily in the fluid itself, and continue to exist in a virulent form in the throats of persons who have been injected with antitoxine. Wassermann [1] claims to have succeeded in the production of a serum which is bactericidal. He prepares a fluid somewhat analogous to Koch's "new tuberculin," by pounding up the bacilli and extracting them with ethylene-diamine, 20 cc. of this solvent being added to 1 gramme of pulverised bacilli. The mixture is well shaken in a special apparatus, and is then submitted to the centrifuge. The supernatant fluid is of a yellow colour, and contains the intracellular toxines of the bacilli. It is capable of killing rabbits when injected into them. If, however, the toxine is mixed with a proportion of antitoxine and repeatedly injected into these animals, the serum obtained from them is strongly agglutinative of diphtheria bacilli. Wassermann thinks that this serum will afford a means of distinguishing *B. diphtheriæ* from pseudo-diphtheria bacilli, and that it may possibly have curative properties.

Concetti [2] writes in favour of the use of antibacterial serum as an adjunct to antitoxine, and also as a local application to the throat, in the form of lozenges. A "bivalent" (antitoxic and antibacterial) serum may also be used prophylactically, as well as for irrigation of the nostrils to avoid spread of infection to the nose; for local application to the trachea after tracheotomy (2·5 cc. every three hours); and in conjunctival diphtheria (Bandi [3]). Dopter [4] advises the local use of antitoxic serum in the form of lozenges; but this serum is theoretically inferior to the bivalent serum. He also uses insufflations of powdered dry serum for nasal

[1] *Deutsch. med. Woch.*, Oct. 30, 1902.
[2] *Riv. di clin. Pediatr.*, 1905, No. 6.
[3] *Il Policlin.*, July, 1906.
[4] *Gaz. des. Hôp.*, 1905, p. 459.

diphtheria and finds this procedure of some value, but less so than in faucial cases.

VACCINE-TREATMENT

Possessing, as we do, in antitoxic serum so potent and reliable a remedy, and one, moreover, which has the overwhelming advantage of being available at a moment's notice, it is not surprising to find that diphtheria-vaccine has not been employed therapeutically to any large extent. At the same time vaccine-treatment is of distinct value in those infections which become chronic, and in which the responsible organism remains domiciled in the throat or nose for prolonged periods after the acute clinical symptoms have been entirely removed by the employment of antitoxine. In such cases a vaccine should be prepared from that strain of *B. diphtheriæ* actually infecting the patient, standardised, and administered in doses of 5 to 10 millions at intervals of five to seven days. One to two injections are usually sufficient to ensure the disappearance of the bacilli from the local site of infection.

AGGLUTINATION

The agglutination of diphtheria-bacilli is not very easily obtained, as the bacteria naturally occur in masses, closely adherent together. Lubowski[1] obtained the bacilli in a state of division by shaking up an emulsion of them with small glass balls, and diluting the resulting fluid with a 10-per-cent. solution of glycerine. The reaction is of no practical value in the diagnosis of diphtheria.

CONCLUSIONS

1. *Prophylaxis.*—Diphtherial antitoxine has a definite power of preventing the onset of diphtheria. The prophylactic dose should be 500 units. Antitoxine should be used with this object in institutions where children are congregated together, if there is any tendency to an endemic

[1] *Zeitschr. f. Hygiene*, 1900, Bd. xxxv.

prevalence of the disease in the institution. In other cases, if the children can be kept under close observation these protective doses are not absolutely necessary, as the prompt administration of the serum on the first appearance of the disease is an almost absolute safeguard against a fatal issue. If "swabs" can be taken from the throats of children exposed to infection, and examined for bacilli, the prophylactic doses of serum need only be given to such as exhibit the organisms in their fauces.

2. *Treatment.*—The curative effects of the serum are well established. The remedy should be given as early as possible in the course of the disease, as the mortality is progressively greater according as the serum is administered on later and later days. Therefore, if the clinical appearances are those of diphtheria, it is advisable to secure material for bacteriological examination, but to *administer antitoxine without waiting for the bacteriological report.* The initial dose for an ordinary case need not exceed 4,000 units. In severer cases much larger doses may be given. The dose may be repeated as often as necessary at intervals of 4, 6, 12, or 24 hours. Serum should be re-administered in case of relapse, but true relapse must be carefully distinguished from simple angina redux.

3. *Method of Administration.*—In ordinary cases subcutaneous injection of the serum is advisable. In very severe cases it may be given intravenously (warmed), in order to get the patient under its influence as soon as possible. The efficacy of the use of antitoxine by the mouth or rectum needs further elucidation. In the present state of our knowledge it is hardly justifiable to make use of this method of administration.

4. Ordinary measures of prophylaxis (isolation, &c.), and of treatment should not be omitted because antitoxine is used.

CHAPTER V

TETANUS

Causal organism.—The micro-organism (*B. tetani*) which is the cause of tetanus was discovered by Nicolaier in 1884, and was first cultivated by Kitasato in 1890.

Entering the body by a wound in the skin or mucous membrane,[1] the bacilli do not become generalised by means of the blood-stream,[2] but remain localised at the point of infection, where they form their toxines. These are taken up by the peripheral nerves, and are carried along the axis-cylinders to the central nervous system. Exactly the same mode of conveyance seems to hold good in the case of the poison of hydrophobia, and it seems that there must be a continual streaming of the protoplasm of the nerve-fibrils towards the cells from which they are prolongations. Resection of a portion of a nerve may prevent the onset of tetanus in animals, if only a very small dose of the toxines has been administered. If large doses are given, part

[1] Commercial gelatine is very liable to contain the spores of tetanus, and a number of cases have been recorded in which the disease occurred after gelatine-injections, made for the treatment of aneurysm. An outbreak also occurred in the United States, due to contamination of diphtherial antitoxine with the toxines of tetanus. Cases have occurred after vaccination, either due to the presence of the organisms or their spores in vaccine-lymph, or to subsequent inoculation of the vaccination-lesions from accidental sources. Vaccination-wounds may perhaps afford a specially favourable nidus for the organisms, as they form an ulcerated surface beneath a scab. Undeserved discredit was thrown upon Haffkine's method of antiplague vaccination by some cases of tetanus arising from neglect of antiseptic precautions in the use of his prophylactic.

[2] Lambert (*Med. News*, 1900, July, p. 12) states that the bacilli may wander into the general circulation.

of the poison is absorbed into the blood-stream, and reaches the nervous system by a more circuitous route. Meyer and Ransom,[1] however, as the result of many ingenious experiments, consider that it is only by the peripheral nerves that the poison can reach the spinal cord or brain. Poison circulating in the blood is taken up by the nerve-endings, and so passed on to the central portions of the nervous system, but it does not penetrate directly into the latter from the lymph. They thus explain the greater part of the incubation-period met with in poisoning by tetanus-toxines. They point out that the incubation is longer in direct proportion to the size of the animal and the consequent length of the nerves. Thus a mouse exhibits symptoms in 8 to 12 hours, a guinea-pig in 13 to 18, a rabbit in 18 to 36, while in man four days is about the shortest period, and in the horse five days. If an animal is injected with the poison in the nerve of a hinder limb, the spread of the poison to the important centres in the medulla can be prevented by section of the spinal cord. In this, as in the peripheral nerves, there appears to be a centripetal movement of protoplasm.

In cases of tetanus in man, the *incubation-period* is very variable. Symptoms may appear in four or five days, or they may be delayed for months. The more rapid the onset, the more acute are the symptoms, and the more grave the prognosis.

Toxines of the tetanus-bacillus.—The toxines of the bacillus may be obtained by growing the organisms in an atmosphere of hydrogen in glucose-broth, from which all oxygen has been expelled by causing hydrogen gas to bubble through it. Sulphindigotate of soda may be added to the broth to act not only as a reducing agent, but also as an indicator, the blue colour disappearing when the last trace of oxygen has been removed from the medium. Marie and Morax [2] state that a potent poison can be prepared by culti-

[1] *Arch. f. exper. Pathol. u. Pharmakol.*, 1903, Bd. xlix., Hft. 6, p. 369. *Cf.* Marie and Morax, *Ann. de l'Inst. Pasteur*, 1902, p. 818; 1903, p. 335.

[2] *Ann. de l'Inst. Pasteur*, 1902, p. 418.

vating the bacilli in association with *Bacillus subtilis* in the presence of air. The fluid obtained after about a month's growth is filtered through a porcelain filter, and the poison is ready for use. The fluid solution does not keep at all well, the poison gradually losing strength, probably owing to the formation of toxones similar to those described in connection with diphtheria (p. 76). The toxines are not destroyed by heating to 135° C. for ten minutes; their action is, however, slightly retarded. Their potency is rapidly destroyed by sunlight. If the albuminous constituents of the culture-fluid are precipitated by alcohol or other reagents, the toxine is found in the precipitate; but it is probably not of an albuminous nature itself. Dean[1] concludes as follows with regard to it:—"Tetanus-toxine has thus many points of resemblance to the soluble ferments; it is difficult to dialyse, is soluble in water, is precipitated by alcohol and tends to adhere to precipitates, is modified or destroyed by the action of air, sunlight, and comparatively low temperatures, and requires an incubation-period for its action."

The mixed poison, as obtained from cultures, is almost certainly very complex in character. Tizzoni and Collina[2] state that it contains two toxines, one of which acts specially if it is administered subcutaneously, and produces convulsions, while the second becomes prominent in case of intravenous injection, giving rise to local tonic spasm. Erhlich[3] has shown that there is also present a substance which produces hæmolysis (*tetanolysin*), and that an antilysin which neutralises this is found in tetanus-antitoxine.

Tetanus-toxine, or *tetanine*, as it is called by French writers—using the word to apply to the poisons collectively—has a great affinity for the nervous system. If

[1] Art. "Tetanus" in Quain's *Dictionary of Medicine*, edited by Montague Murray, 1902, p. 1688.

[2] *Gazz. degli Ospedali*, 1901, No. 138.

[3] Quoted by Dean, *loc. cit.*

an animal has died of tetanus, the nerves leading from the seat of infection, and also the brain and spinal cord, contain the poison. The affinity of the brain-tissue for the toxine has also been shown in another way by Wassermann and Takaki,[1] who found that if an emulsion were made of brain-substance, and this were mixed with the toxines before injection into animals, no ill effects were produced. A protective influence was exercised even if the emulsion were injected at a different point from the toxine, without previous mixture. Cerebral substance seems thus to contain an antitoxine to the poison : in other words, the toxine has the power of combining with the side-chains of the cerebral cells ; when, therefore, these cells are injected into another animal, they are capable of uniting with the free poison and so preventing it from attacking the living tissues of the animal.

The pathological effects of tetanus are seen in the cells of the nervous system, and consist in swelling of the chromatic bodies and cell-body, followed by progressive chromatolysis. These lesions are of rather irregular distribution, being more uniform and intense in the brain than in the cord ; and with the possible exception of the early stages of the process, the changes are not specific of the disease.

The toxine exists in other organs besides the nervous system, and may be extracted from them by glycerine.[2] The peripheral nerves contain it, but have not the same neutralising action as is possessed by the brain and spinal cord. The occasional appearance of a peripheral neuritis in patients who recover from tetanus has been attributed to a toxone analogous to that of diphtheria.[3]

TETANUS-ANTITOXINE

Preparation of antitoxine.—For the practical preparation of antitoxine for tetanus, horses are used. These

[1] *Berlin. klin. Woch.*, 1898, No. 1.

[2] Waring, *Proc. Path. Soc. Lond. ; Brit. Med. Journ.*, 1902, i. 965.

[3] Grünberger, *Wien. klin. Woch.*, 1904, 737.

animals are very sensitive to the toxines of the disease, and great care is necessary in the process of immunisation. At the beginning of the treatment use is made of a toxine which has been attenuated by means either of heat or of some chemical agency. Iodine trichloride is used by Behring. After the blood of the animal has been found to contain a considerable amount of antitoxine, as a result of these injections, the undiluted toxine may be administered. The method of procedure is the same as that already described in the case of diphtheria.

Standardisation of antitoxine.—A method of standardising tetanus-antitoxine similar to that in use for anti-diphtherial serum has been introduced by Behring. A test toxine is prepared of such a strength that 0·01 cc. will kill a guinea-pig of 500 grammes in about four days. This amount of toxine is neutralised by $\frac{1}{1000}$ of a unit of antitoxine. In other words, one unit of antitoxine will protect 1,000 guinea-pigs against the minimal lethal dose of toxine.

According to Roux's method of standardisation, the value of the antitoxine is expressed according to the amount of guinea-pigs (calculated in grammes) which 1 cc. of the serum will protect from a minimal fatal dose (for that number of guinea-pigs). Thus if 1 cc. of a certain serum will protect 100 guinea-pigs, each weighing 500 grammes, against the minimal amount of toxine which would otherwise kill them in four days, the value of the serum is said to be 50,000 (100 × 500). This would be a very weak serum for use, 1,000,000 units per cc. being an average strength.

Rosenau and Anderson[1] take as a unit of antitoxine ten times the least quantity of antitoxine which will keep a guinea-pig (of 350 grammes) alive for 96 hours after injection of an official toxic unit. This unit is the equivalent of 100 minimum lethal doses.

Unfortunately no standard strength is adopted in the

[1] *Bull. No.* 43, *Hyg. Lab. U.S. Pub. Health and Mar. Hosp. Service,* 1908.

serums on the market. The dose is generally calculated in cubic centimetres, without any statement of the number of units contained, so that not only is accurate dosage impossible, but cases recorded as treated with antitoxine have very little value, owing to the impossibility of knowing how many units of antitoxine were really used.

Experimental value of antitoxine.—From experiments made in the laboratory, in which all the factors are under control, there can be no doubt that tetanus-antitoxine, if given along with, or shortly after, a dose of the toxine, has the power of preventing the occurrence of the characteristic symptoms and of death. Dönitz[1] finds that, whereas a certain dose of antitoxine suffices to neutralise a definite quantity of the toxine when it is injected at the same time, if a space of four minutes is allowed to elapse between the administration of the toxine and that of the antidote, then a slightly larger amount of the latter is needed. If eight minutes intervene, then six times the original neutralising dose of antitoxine is required; if sixteen minutes, then twelve times the dose; and at the end of one hour, twenty-four times the amount of antitoxine is requisite. Here, as in the case of diphtheria, a "mass" action of the antitoxine may be exerted for a certain time after the poison is injected; but there comes a time in both cases in which this is no longer possible—when the toxine has entered so closely into combination with the cells that no amount of antitoxine is capable of withdrawing it. After that time even the minimal lethal dose is of necessity fatal. If the blood of an animal is rendered antitoxic to tetanine, injection of this poison into the substance of its brain will still produce a fatal effect as surely as if no protective power had been gained.

General considerations on the use of antitoxine.—Tetanus is a rare disease in this country, and is comparatively uncommon in most civilised parts of the world. Hence statistics are difficult to collect, and are

[1] *Deutsch. med. Woch.*, 1897, p. 430.

generally founded on insufficient numbers of cases for accuracy. Further, the disease may occur in an acute or in a more or less chronic form, these varieties merging one into the other with no distinct dividing line. The mortality in chronic cases is much less than in the acute. Owing to these peculiarities, it is difficult to calculate what the mortality from the disease was before the introduction of antitoxine, and the statistical method, such as was used for demonstrating the value of diphtherial antitoxine, is here even more liable to be vitiated by errors of observation.

In recorded cases of tetanus it is generally impossible to gather a definite opinion as to the severity of the case, and hence it is difficult to realise what would have been the chance of recovery without the use of antitoxine; while a comparison of a small series of six or eight cases with a theoretical general-mortality figure, of the vague description which we have just indicated, gives very little information. On the other hand, the observer's opinion in any individual instance, as to the effects produced by the remedy in abating symptoms or conducing to recovery, is unreliable.

The data obtained by means of experiments on animals, as to the behaviour of the poison in the body and as to the neutralising effects of the antitoxine, afford very clear indications as to what we may expect from the latter in the treatment of the disease. Nothing is more definitely established than that there comes a period of time at which the toxine is so closely attached to the cells of the nervous system that no amount of antitoxine will suffice to withdraw it or to counteract its effects. This, unfortunately, occurs very early in the disease; indeed, the vital question is, whether it is not already too late for the antitoxine to have any good effect when the disease has declared itself, *i.e.* when the symptoms, by which alone the infection can be recognised, have appeared.

It does not, however, follow that the remedy is of no effect at all. It is impossible to know in any given case whether the amount of toxine which has been taken up by

the nerves is sufficient to cause a fatal termination. By administering antitoxine we cut off the supply of the poison and prevent further absorption. It might be that a quantity of toxine just short of the minimal fatal dose had been absorbed at the time when the case came under treatment. If the remedy were given at once, the further absorption of the poison would be stopped, and the balance would be turned in the patient's favour; whereas if any delay in giving the remedy were allowed to arise, time might be afforded for the absorption of the additional amount of toxine necessary to cause death. Hence theoretical reasons lead us to the conclusion that the antitoxine should be used in all cases directly they come under treatment, but that no great fall in the mortality is to be expected to result. A few cases only will be saved; no brilliant statistics of cures are to be looked for; but we can never know, till death has actually ensued, that the case before us is not one of the exceptions in which the use of antitoxine will turn the scale towards recovery.

Statistics of the use of antitoxine in tetanus.—We have already pointed out that tetanus is too rare a disease for the statistics of it to be valuable; further, the use of antitoxine is of comparatively recent introduction, so that no large number of cases are available for the study of its effects. Lambert[1] collected 262 cases, of which 151 recovered, giving a total mortality of 42·36 per cent. These were divided into 124 acute cases, of which 35 recovered, giving a percentage mortality of 71·77; and 138 chronic cases, of which 116 recovered, leaving a mortality of only 15·94 per cent. On these figures he pronounces strongly in favour of the use of the remedy, especially in the chronic cases, in which the previous mortality was about 40 per cent.; in these his figures show a reduction in mortality (due to antitoxine) of approximately 24 per cent. Abbe[2] saw six cases of tetanus in pre-antitoxine days, of which only one recovered; whereas of nine others treated with

[1] *Op. cit.* [2] *Ann. of Surg.*, 1900, xxxi., 273.

this remedy seven survived. Five of these latter cases were severe in type, and of them three recovered. For reasons already given it does not seem worth while to set forth in detail the figures given by different writers. Rozenraad[1] quotes the following records: Engelmann, 54 cases treated with antitoxic serum, mortality 28 per cent.; Lund, 167 cases, mortality 35 per cent.; and Kochler, 96 cases, mortality 34·4 per cent.

Busch[2] collected 147 cases with a mortality of 44 per cent. Schley[3] quotes Packard and Wilson's figures, viz. 1,216 cases with a mortality of 42 per cent., and Moschewitz's 461 cases with mortality 40·3 per cent. It is impossible to say how far these various collections of cases overlap one another.

Mode of administration.—*Subcutaneous* injection of antitoxine was that first employed in tetanus, as in diphtheria, and the majority of the cases on record have been treated by this method. It was, however, pointed out that the antitoxine was only slowly absorbed by this route, whereas it was important to neutralise the toxine, which had already got the start of the antidote, as quickly as possible. Hence *intravenous* injection has been advised as a speedier method. It certainly seems to be preferable to the subcutaneous route.

Both of these methods effect a neutralisation of the poison circulating in the blood, but they do not avail to counteract that which has already reached the nervous system. Two methods adapted to achieve this end, if it be possible in any way, have been recommended, namely, the injection of the antitoxine into the space between the dura mater and the brain or spinal cord, and injection directly into the brain-substance. The former is known as subdural, the latter as intracerebral injection. Both of these have their advocates.

[1] *Quinzaine Thérapeut.*, 1904, p. 63.
[2] *Arch. f. klin. Chir.*, 1907, lxxxii., Heft 1.
[3] *Med. Record N.Y.*, 1904, p. 616.

In favour of the *subdural* method, Leyden[1] points to the results of eleven cases which he has collected, among which there were six deaths and five recoveries. Two of the latter were cases of his own. It is certain that the cerebro-spinal fluid contains toxine in fatal cases of tetanus, and the subdural injection will neutralise this. It is, however, uncertain whether the toxine is taken up by the central nervous system directly from the fluid in which it is bathed. Meyer and Ransom, as already quoted, do not think that it is; but there is no doubt that other substances, such as cocaine, are thus absorbed. Further experiment seems necessary to decide this question positively.

Jacob[2] states that he was successful in saving two-thirds of his cases at the Charité Hospital in St. Petersburg which were treated by the subdural method. He withdraws 10 cc. of cerebro-spinal fluid by lumbar puncture, and then slowly injects 10 to 20 cc. of antitoxine. Penna[3] gave the remedy beneath the cerebral membranes, using large quantities of serum—in one case 60 cc. at a dose. Daily injections were given, amounting in all to 100–240 cc. per case. Of five patients, three recovered and two died—the latter of intercurrent pneumonia, not of tetanus. Wallace and Sargent[4] report four cases treated by injection into the spinal theca; of these three recovered, one being a severe case and two subacute.

Theory is very strongly in favour of the method of *intra-cerebral* injection. It has been pointed out that if the toxine is injected directly into the brain, no amount of anti-toxic power in the blood will avert a fatal issue. The toxine appears to be passed on from one cell to another in the central nervous system, and it is to be supposed that

[1] *Deutsch. med. Woch.*, 1901, No. 29, p. 477.

[2] *Russky Vratch*, Jan. and Feb., 1902 (abstr. in *Journ. of the Amer. Med. Assoc.*, 1902, i. 977).

[3] *Semana Medica*, Oct. 31, 1901 (abstr. in *Journ. of the Amer. Med Assoc.*, 1902, p. 602).

[4] *Lancet*, 1904, i. 642. *Cf.* Neugebauer, *Wien. klin. Woch.*, 1905, No. 18.

the antitoxine will also be thus diffused. Roux[1] saved 35 out of 45 guinea-pigs inoculated with tetanine by this method, whereas by the subcutaneous injection he had only two recoveries out of 17 animals. Letoux[2] records four cases of recovery in human patients after intracerebral injection. He administered 10 cc. of antitoxine into each hemisphere.

Abbe[3] speaks in favour of the intracerebral route. The procedure adopted is as follows: A line is drawn from one auditory meatus to the other across the vertex of the skull. From the point at which this meets the mid-line a second line is drawn to the outer angle of the orbit. The middle point of this last line gives the site for the injection (*Roux's point*). An incision is made in the scalp, and a small trephine is employed to remove a piece of the skull. Abbe says that the operation can be done satisfactorily under cocaine-anæsthesia. There does not seem, however, to be any advantage in this over chloroform, as the latter will control any spasm which may occur during the operation, while cocaine will not. An opening is made in the dura mater and a blunt needle is thrust into the cerebral substance. The antitoxine must be very slowly forced into the brain, 5 cc. being enough to use on each side. It would seem better to endeavour to inject the antitoxine into the actual brain-substance than into the lateral ventricle,[4] as the latter method is practically equivalent to subdural injection, which can be more easily carried out, if it is desired, externally to the brain. A procedure similar to the above may be adopted for subdural injection, if it be decided to give this intracranially, but the method by spinal puncture is in all probability as effective. An actual trephine-opening in the skull is not necessary for the intracerebral injection, which can be given through a simple hole

[1] Quoted by Abbe, *op. cit.*

[2] *Semaine Méd.*, 1901, p. 349.

[3] *Op. cit.*

[4] *See* Kocher, *Centralbl. f. Chirurg.*, 1899, No. 22, as to technique.

bored with a drill. The large cerebral sinuses must, of course, be avoided in these procedures, and a blunt needle is preferable, in order to avoid wounding the smaller vessels in the substance of the cerebrum.

For the purpose of intracerebral injection it is advisable to prepare a specially strong serum[1] by dissolving the solid (dried) substance in half the usual quantity of distilled water, or by evaporating the liquid serum to half its bulk at a low temperature *in vacuo*.

Intraneural injection has also been practised, in view of the supposed route by which the poison is absorbed. Good results are recorded in individual cases.[2] It is best to make use of the subdural method in addition.

Behring[3] considers that no good results are to be hoped for from the use of antitoxine if it is administered more than 30 hours after the onset of symptoms, or if less than 100 units (on his system) are given. According to the experience of Moeller,[4] even if these postulates are fulfilled, no great fall in the mortality is to be looked for.

A considerable number of isolated cases are reported, but as they are by different observers, using different brands of serum and employing a variety of methods, little would be gained by tabulating them. Tizzoni[5] considers that the serum which he prepares is superior to that of Behring, while German writers[6] apparently hold that the disease in Italy is less virulent than in their own country. No exact data seem to be available for estimating the truth of these international amenities.

On the whole, it must be confessed that, as was to be

[1] Church, *New York Med. Journ.*, Dec. 17, 1898.

[2] *See* Rosenraad, *Quinzaine Thérapeut.*, 1904, p. 63; Apert and L'Hermitte, *ibid.*, 199; Sicard, *ibid.*, 199; Clairmont, *Wien klin. Woch.*, 1905, No. 49; Rogers, *Med. Record*, 1904, lxv. 813, and lxvi. 12.

[3] *Deutsch. med. Woch.*, 1900, No. 2.

[4] *Ibid.*, 1901, No. 47, p. 814.

[5] *Riforma Medica*, 1901, i. 366.

[6] *See* Pfeiffer, *Zeitschr. f. Heilkunde*, Bd. xxxiii., Heft 2.

anticipated on theoretical grounds, the curative treatment of tetanus by antitoxine is disappointing, as compared, at least, with the results obtained in diphtheria. Nevertheless, it is our duty to give the remedy a trial in all cases, since it is impossible to be sure that it will not do good, and it can in any case do practically no harm.

Prophylactic use of antitoxine.—There is hope that antitoxine will prove of greater service by way of prevention than as a cure for the developed disease. Experiments on animals point strongly in this direction. Actual results are also encouraging. In veterinary practice some striking statistics are quoted by MacFarland.[1] In one of the large factories for the production of antitoxine (diphtherial) much trouble was at one time caused by the incidence of tetanus among the horses. At last systematic use of prophylactic injections of antitoxine were instituted, and the result was that, whereas in one year (1898) the death-rate among the animals was 10 per cent., in the year following it fell to 1 per cent., and in the year after that to a mere fraction. Nocard[2] reports that he injected 2,727 horses with antitoxine in a certain district; none of them developed tetanus, although among other animals in the same part of the country there were 259 cases of the disease. Herhold,[3] who was surgeon to the German contingent on the expedition to Pekin, at first lost several patients by tetanus. Afterwards he used prophylactic injections in all cases of crushed wounds contaminated with dirt, and no more cases of the disease occurred. Fisch[4] considers that the preventive use of the antitoxine was of considerable service in the epidemic which occurred at St. Louis from contaminated diphtherial antitoxine.

At the same time, it seems necessary to admit that an injection of antitoxine is not a certain preventive of tetanus,

[1] *Journ. of the Amer. Med. Assoc.*, July 4, 1903, p. 34.
[2] Quoted by Lambert, *op. cit.*
[3] *Deutsch. med. Woch.*, 1901, No 30, p. 479.
[4] *Interstate Med. Journ.*, Dec., 1901.

as Reynier[1] records that in a hospital in Paris, in consequence of an outbreak of the disease, a patient was given a prophylactic injection. In spite of this she developed tetanus, though she finally recovered. Two other patients died of the disease; so it is possible that even here some good was done, and what would otherwise have been a fatal attack was rendered milder.

We may conclude that in countries where tetanus is a comparatively common complication, all cases of crushed or dirty wounds should receive a prophylactic injection of antitoxine. How long the protection will last is not known. As the serum, being derived from the horse, is exactly analogous to diphtherial antitoxine, it seems legitimate to assume that it will be excreted or neutralised at the same rate as the latter; hence we may conclude that the protection will remain effective for about three weeks, but Dehne and Hamburger[2] believe that it ceases after about one week.

Dose of antitoxine.—As was mentioned above, records of cases generally state the number of cubic centimetres of serum injected. A dose is usually from 10 to 20 cc. The amount of antitoxic units contained in the serum is not often noted on the bottle, and hence is not known to the administrator. This is unfortunate, as no accurate dosage or comparison of results is possible. Behring advises that not less than 100 of his units should be given at once. Amounts of 10 to 20 cc. are easily administered subdurally, around either the spinal cord or the brain. Three to five cubic centimetres can be injected into the substance of the brain by slow instillation. Prophylactically, 10 cc. may be given.

Ill effects of antitoxine.—The same by-effects may be expected to occur after the use of tetanus-antitoxine as after antidiphtherial serum, since it is horse's serum which

[1] Quoted by Moeller, *Deutsch. med. Woch.*, 1901, No. 47, p. 814.

[2] *Wien. klin. Woch.*, 1907, Nos. 1–3

is used in both cases. Di Gaspero[1] describes a case in which a fatal issue occurred, due, as he thinks, to serum. This inference is very doubtful. Rashes of various kinds, scarlatiniform, bullous, urticarial, &c., are also recorded.

TREATMENT WITH CEREBRAL EMULSION

In view of the great affinity of the substance of the central nervous system (brain and spinal cord) for tetanus-toxine, use has been made of emulsion of fresh brain-substance as treatment for the disease Krokiewicz[2] records 16 cases in which use was made of this preparation, an unfiltered emulsion being injected hypodermically. Of the 16 cases, 13 recovered, three of them being severe attacks.

When we consider the difficulty with which an emulsion of cerebral substance must be absorbed and reach the circulation, it is easy to realise that the curative action of such a remedy must necessarily be slower than that of antitoxic serum. The latter should therefore have the preference, if it be available. If, however, no antitoxine be at hand, it would certainly be not only legitimate but also advisable to make trial of cerebral emulsion. The mode of action of the two remedies is theoretically the same, the receptors of the cerebral cells being available in the emulsion to anchor the toxine and neutralise it, just as the free receptors (side-chains) do in the antitoxine. The diffusion of the emulsified cells must be less rapid than that of the cast-off receptors—which are presumably separate molecules, not whole cells.

CONCLUSIONS

1. Tetanus-antitoxine cannot be expected to *cure* any large proportion of patients in whom the disease has developed. It should, however, be given in all cases of

[1] *Die Therap. der Gegenwart*, 1902, p. 139.
[2] *Klin. therap. Woch.*, Feb. 8, 1903.

tetanus, since it may just turn the scale in the patient's favour by neutralising the poison circulating in the blood, although it may not reach that already absorbed by the nervous system.

2. The *dose* should be from 10 to 20 cc. if the strength of the serum is not stated. If this be known, not less than 100 units (Behring) should be administered at once. The injection may be repeated as often as seems necessary, at intervals of 6 to 12 hours.

Intracerebral injection probably offers the best chance of neutralising the poison already absorbed ; this method should be used in severe cases, intravenous or subcutaneous injections being employed at the same time.

In less severe cases intraspinal injection through a lumbar puncture (about 10 cc. of cerebro-spinal fluid being first withdrawn) should be practised, immediately followed by intravenous injection.

3. In countries where tetanus is common, *prophylactic* injections of antitoxine should be used in all cases of crushed wounds in which dirt or other foreign matter has been ground into the wound. The antitoxine may be given subcutaneously in such instances.

CHAPTER VI

SNAKE-BITE

Classification of snakes.—The poisonous varieties of snakes belong chiefly to the families *Colubridæ* and *Viperidæ*. The best-known kinds belonging to the Colubrine group are the Cobras (*Naja*), the Coralline snakes (*Elaps*), the Kraits (*Bungarus*), and the Death-adder (*Notechis*). To the Viperine division belong the Common Viper (*Vipera berus*), Russell's Viper (*Daboia*), the Puff-adder (*V. arietans*), the Rattlesnakes (*Crotalus*), the Bush-master (*Lachesis*), the Tiger-snake (*Hoplocephalus*), and the Copperhead (*Denisonia*). The poisonous water-snakes (*Hydrophinæ*) are classed as Colubrine snakes. The classification depends mainly on small differences in the teeth and the bones of the head.[1]

The amount of poison injected in an individual case depends on the efficiency with which the stroke was delivered, while the virulence of the poison varies with the health and vigour of the snake at the moment, and to some extent with the season of the year. Calmette believed that a full-sized cobra would inject at one stroke about 30 to 45 milligrammes of venom ; but Cunningham puts the quantity at 254 mgr., and Rogers at 249 mgr. Lamb reckons the average amount as 200 mgr.[2]

Different animals vary somewhat in the degree of suscep-

[1] An account of the varieties of poisonous snakes may be found in Calmette's "Venoms, Venomous Animals and Antivenomous Therapeutics." Trans. by Austin, London, 1908.

[2] *See* Rogers, *Ind. Med. Gaz.*, Sept., 1904, p. 332 ; Lamb, *Glasgow Med. Journ.*, Feb., 1903, p. 85.

tibility which they present to the action of snake-poison. Rabbits, guinea-pigs, and herbivorous animals in general seem more susceptible than carnivora, such as the cat or the dog. Man is probably about equal in this respect to the latter class. The minute dose of poison which is capable of causing symptoms is illustrated by the experience of the late Mr. Frank Buckland, who accidently pricked his finger in the process of dissecting the body of a rat killed by the bite of a poisonous snake. Only an infinitesimal amount of fluid could have been inoculated, and the original dose of poison had been diluted by diffusion throughout the body of the animal ; nevertheless, alarming symptoms of faintness and collapse ensued.

Nature of the poison.—The poison may be collected by killing the snake and dissecting out the gland and receptacle ; or the snake may be "milked" by pressure on the sac ; or it may be made to bite on a watch-glass covered with an indiarubber membrane, and the poison thus collected in the glass. The last method is probably the best. The poison is dried *in vacuo*, and may be preserved indefinitely in this condition.

It has long been known that the poisons of different snakes are not identical in their effects ; the poisons of the viperine group having, as a rule, a more intense local action, those of the colubrine family rapidly producing a general intoxication. Calmette, however, to whom we are indebted for the original preparation of an anti-venomous serum (*antivenene*), considered that the main toxine, at all events, was the same in all venoms, and could be counteracted by one and the same antitoxine. This view was combated by Martin, who, as the result of his experiments, came to the conclusion that there were two substances present in the venom of all snakes, varying, however, in proportions in different species. One was a globulin, coagulable by heat ; the other a peptone, which was resistant to it. The former is responsible for the general nervine intoxication, the latter for the local effects and blood-changes.

More recently Lamb[1] has given reasons for rejecting these views. He is inclined to think that the poison of each genus of snakes is specific, containing a kind of poison not met with in other varieties. Thus he holds that though the poisons of the cobra and the daboia are each complex, no single constituent is common to both. The reasons for this will be clearer when we come to consider the action of antitoxic serum as an antidote to the poisons of different snakes. Lamb originally considered that in all probability the poisonous element was not proteid in nature. He showed that the proteins in the different venoms are, as far as can be determined, the same in all, while the effects produced are diverse. This would bring snake-poison into the same category as the toxines of diphtheria and tetanus, of which the former was shown to be developed in a non-albuminous medium, while the latter, according to Dean, is also of the nature of a ferment rather than an albumin. Lamb[2] appears, however, to have now changed his opinion and believes in the proteid nature of the poison. Faust,[3] on the other hand, states that he precipitated a poison (*ophiotoxine*) five times as toxic as crude venom, from cobra-venom by means of alcohol after all the proteins had been removed.

Action of venom.—Locally, the effects produced by snake-bite are swelling, redness, and ecchymosis; if the patient survive the first effects, cellulitis and sloughing of the parts may occur, and the œdema may spread up the affected limb. The general effect of the poison is shown in its action upon the nervous system, which takes the form of depression, collapse, nausea or vomiting, incoördination, paralysis, and convulsions, ending in coma and death by asphyxia or heart failure. Hæmorrhagic discharges from mucous membranes are sometimes seen.

[1] "Scientific Memoirs by Officers of the Med. and Sanit. Depts. of the Gov. of India." No. 5, "Specificity of Anti-venomous Sera," 1903.

[2] *Glasgow Med. Journ.*, Feb., 1903, p. 86.

[3] *Arch. f. exp. Path.*, 1907, lvi. 236.

Experimentally, snake-poison is found to have a solvent action on the red corpuscles of the blood (hæmolysis). Lamb finds that both cobra- and daboia-venom have this property. Cobra-poison, however, acts more powerfully outside the body than within it, while daboia-venom is more hæmolytic *in vivo* than *in vitro*. The hæmolytic substance in cobra-venom is not coagulable by heat. Rattlesnake-venom is attenuated by heat and by the action of hydrochloric acid. Cobra-hæmolysin is also weakened by heat, but recovers its virulence on standing.[1] Flexner and Noguchi[2] assert that the hæmolytic agent in venom is of the nature of a copula or intermediary body (*see* p. 8). The complement which is necessary to complete its action on the blood-corpuscles is contained within the corpuscles themselves (endo-complement). This view has been confirmed by Kyes,[3] who was successful in preparing a chemical compound resulting from the union of the copula and lecithin. This he named "cobra-lecithide." It has a direct hæmolytic action. Snake-poison also possesses the power of agglutinating red corpuscles, the clumps thus formed being disintegrated again by the action of permanganate of potassium. The agglutinative power is removed by heating the venom to 75° or 80° C., whereas the hæmolytic power still remains after this treatment, showing that the two properties are dependent on distinct toxines. Leucocytes are destroyed by the poison as well as red corpuscles (leucolysis). The different kinds of leucocytes contained in rabbit's blood are unequally affected by the leucolytic substance, the lymphocytes being the least susceptible.

The ecchymoses seen in cases of snake-bite are due to the action of the poison on the endothelial cells forming the lining membrane of the capillaries. This action is

[1] *See* Flexner and Noguchi, *Journ. Med. Research*, 1904, vi. 363; Morgenroth and Pane, *Biochem. Zeitschr.*, 1906, i. 354.

[2] *University of Pennsylvania Medical Bulletin*, 1902, xiv. 438; and xv. 345.

[3] *Berlin. klin. Woch.*, 1903, Nos. 42, 43.

analogous to hæmolysis, and is due to a destructive "lysis" of these cells by a special substance, which is called by Flexner and Noguchi "hæmorrhagin," from its effect in producing hæmorrhages. It is suggested by these observers that possibly similar substances may exist in the blood of patients suffering from the various forms of purpura. These authors further state that venom has the power of dissolving other cells besides blood-corpuscles (*e.g.* liver-cells, spermatozoa, &c.). Daboia-venom also liquefies gelatine by means of a special ferment.

According to Lamb [1] the venom of the daboia produces intravascular clotting of the blood, whereas cobra-poison. on the other hand, has an exactly opposite effect, causing a diminution in the coagulability of the blood. Death in cases of bite by the daboia results from the extensive character of this clotting. Curiously enough, it appears that a small dose of the poison, insufficient to cause this phenomenon, is followed by a diminution of coagulative power; and if this has once been produced, no subsequent injection of further doses of the poison will any longer produce clotting. This peculiar phenomenon is not reproduced *in vitro*, and is dependent on some obscure vital action. The substance in cobra-venom which reduces the coagulative power of the blood is not a protein coagulable by heat. The addition of snake-venom to blood has the effect of reducing the bactericidal power of the latter by depriving it of the necessary complements, which become fixed to the copulas present in the venom. The latter does not contain complement.

The element in venom which acts on the nervous system is called by Flexner and Noguchi "neurotoxin." It, too, is of the nature of a copula, and acts by fixing a suitable complement to the nerve-cells. These observers also found that the brain was the organ in the body which contained

[1] "Scientific Memoirs of the Government of India," No. 4, 1903, "On the Action of the Venoms of the Cobra (*Naja tripudians*) and of the Daboia (*D. Russellii*)," &c.

the most neutralising substance for venom, *i.e.* that its cells have the greatest affinity for the toxine. An animal injected with a minimal lethal dose of venom mixed with emulsion of brain lived many hours longer than one which received the same quantity of poison in blood-serum or similar fluid. After death acute degeneration of the nerve-cells (chromatolysis) is found (Hunter[1]). Rogers[2] states that the poison of the sea-snake, *Enhydrina*, has an action resembling that of curare.

The principal poisonous substances in snake-poison are therefore (1) hæmolytic, (2) leucolytic, (3) hæmorrhagic, and (4) neurotoxic. The bodies having these separate actions are probably different in the various kinds of poisonous snakes. Hence it is evident that the term "snake-poison" or venom includes a very complex group of chemical substances.

ANTIVENENE

Preparation of antivenene.—The possibility of preparing an antitoxic serum (*antivenene*) for the treatment of snake-bite was first practically demonstrated by Calmette, of Lille. His serum is manufactured by injecting a horse with gradually increasing quantities of a mixed venom, containing 80 per cent. of cobra-poison and 20 per cent. of viperine venom. The mixture is heated before the injections are given, as the crude poison is so intensely toxic that the horses are often killed by the minute quantities used for immunisation. Thus MacFarland[3] states that he lost two out of three horses in which he practised the inoculations. It is therefore necessary to proceed with the greatest caution in these injections. A further difficulty is met with in the process, owing to the need of procuring large quantities of venom for the later injections —a need which is not easily satisfied, for obvious reasons.

[1] *Glasgow Med. Journ.*, Feb., 1903, p. 98.

[2] *Lancet*, Feb. 6, 1904, p. 349.

[3] *Journ. of the American Med. Assoc.*, 1901, xxxvii., 1597.

It is advisable to administer to the horse before inoculation one or more protective doses of antitoxine, in order to enable it to withstand the first injections of the poison. Otherwise the method adopted is practically the same as that already described for the antitoxines of tetanus and diphtheria. Tidswell [1] took more than three years in immunising a horse against the venom of the Australian tiger-snake, owing to a combination of the above-mentioned difficulties.

Action of antivenene.—Calmette claims that the antivenene which he prepares is capable of neutralising the effects of the venom of any snake, whatever the species to which it belongs. The mixture of venoms which he uses for the inoculation is designed to render the horse resistant to the poisons of viperine as well as colubrine snakes, even if it be not the case, as he apparently holds, that the venoms of all kinds of snakes are identical. It has, however, been pointed out by Hanna and Lamb [2] that the heating process to which the mixed poison is subjected before injection, in order to render it less virulent, is capable of destroying the potency of viperine poison altogether, or almost entirely. Hence only the cobra-venom is actually left to immunise the horse.

Experience seems to confirm this view to a great extent. Thus Tidswell finds that Calmette's serum has no effect in neutralising the poison of the Australian tiger-snake (*Hoplocephalus curtus*); nor had an antivenene prepared from the venom of this snake any antidotal power against the poisons of other snakes met with in the same continent (*Echis*, *Notechis*). Lamb similarly found that cobra-antivenene has no antitoxic power against the bite of the daboia; and in a later memoir he pointed out that it was unavailing against the poisons of the snakes known as *Bungarus cæruleus* and *Echis carinata*. Serum prepared with the venom of the hoplocephalus by Tidswell had no

[1] *Australasian Med. Gaz.*, April 21, 1902.
[2] *Journ. of Pathol. and Bacter.*, 1902, viii. 1.

neutralising effect upon the venom of bungarus, cobra, or daboia, and an anti-crotalus serum failed to influence the course of symptoms produced by the venom of other snakes (Noguchi). On the other hand Korbel[1] found antivenene useful in the bites of vipers (*V. aspis*, *V. berus*, *V. ursini*, *V. ammodytes*), and Rogers[2] found that it neutralised the venom of the King-cobra (Hamadryas), of the Krait (Bungarus), and of the sea snake (Enhydrina). Martin and Lamb[3] as the result of a study of many venoms and antivenenes conclude that "antivenomous serums are highly but not strictly specific."

These results are of considerable practical importance. If the venoms of the different snakes are thus specific in nature, so that a serum prepared from one of them has no neutralising effect on poison derived from another species, the question of the practical therapeutics of snake-bite becomes much less simple than was originally hoped by Calmette. It would seem necessary to have at hand in all cases a supply of serums for all the different varieties of snakes found in any district, or else to prepare a polyvalent serum by injections of the poisons of all of these reptiles. It does not seem to be known at present how far the latter suggestion is feasible. On the other hand, it is evident that if separate serums were prepared for each kind of snake, it would often be necessary to inject all of them in a case of snake-bite, since the patient could not be expected to know what was the kind of snake which bit him. Bites from snakes often occur at night, when it would be impossible for anyone to identify the assailant; while those who are not skilled biologists would in any case not be likely to know one poisonous variety from another.

With regard to the neutralising action of antivenene on the different constituents of snake-poison, there seems some

[1] *Wien. med. Woch.*, 1908, p. 399.

[2] *Indian Med. Gaz.*, Sept., 1904, p. 332.

[3] Allbutt and Rolleston, *System of Medicine*, vol. ii., Part 2, 1907, p. 815.

divergence of opinion. Flexner and Noguchi[1] state that antivenene is capable of inhibiting the effects, not only of the neurotoxine or main poisonous element, but also of the hæmolytic and other materials. On the other hand, MacFarland[2] finds that it is very difficult, if not impossible, to produce immunity to the local irritation of the poison. He considers, however, that the remedy should be used in all cases, as the counteraction of the most deadly toxine allows the body to concentrate all its resisting powers on repelling the local irritant. According to Auche and Vaillant-Hovius[3] the presence of antivenene does not prevent hæmolysis altogether, but renders it less intense and more transitory. If the neural toxine of snake-poison be removed, the body which gives rise to coagulation of the blood may still cause death, if it is present in a sufficient amount.

Noguchi[4] has prepared an antivenene against rattlesnake-bite (anti-crotalus serum), and Vital[5] prepares two serums, one against crotalus and the other against bothrops, and mixes them to form a polyvalent remedy.

Standardisation of antivenene.—For the purpose of experimental study of the action of venom and of antivenene on animals it is necessary to arrive at some standard of virulence and protective power respectively. This is done by determining the minimal lethal dose for a certain kind of animal (rabbit or rat), calculating the weight of the latter in grammes. Thus Lamb found that 0·05 milligramme (0·00005 gramme) of the venom of *Echis carinata* per kilogramme of body-weight was fatal to rabbits; in other words, a rabbit weighing 1,000 grammes would be killed by the above quantity of poison, while one weighing 1,500 grammes would require half as much again. Of the venom of *Bungarus fasciatus* 0·7 mg. was required to produce the

[1] *Journ. of Experimental Medicine*, March 17, 1902.
[2] *Op. cit.*
[3] *Arch. de Méd. Expérimentale et d'Anat. Pathol.*, 1902, xiv. 221.
[4] *Univ. Penna. Med. Bull.*, Aug., 1904, p. 154.
[5] Quoted by Alabrese, Abstr. *Centralbl. f. inn. Med.*, 1907, p. 152.

same effect. The venoms of different snakes thus differ markedly in their actual toxicity. Further, as was previously stated, different species of animals vary somewhat in their susceptibility to snake-poison, while the actual toxines are probably very different in the various kinds of snakes. Hence no accurate measurement of toxines and antitoxines applicable to all can possibly be arranged.

The only antitoxic serum on the market is Calmette's antivenene, which is effective for cobra-poison. This is standardised by experiments on rabbits. The amount of serum which will protect a rabbit weighing 2,000 grammes against the smallest amount of the toxine that would otherwise kill it, is said to contain 2,000 units of antitoxine. The whole matter is as yet in so experimental a stage that the standardisation of the antivenene is scarcely of practical therapeutic importance.

Dose of antivenene.—Doses of 10 to 20 cc. are generally administered. Lamb, however, considers that this amount of a serum of the ordinary strength is not sufficient to protect against the whole amount of poison which a full-grown, healthy snake can inject at a bite, and he advises the use of not less than 40 cc., if this quantity be available. Rogers calculates that as much as 400 to 800 cc. may be required. If any time has elapsed since the bite, the remedy should be given intravenously. If the case be seen at once, injection into the neighbourhood of the bite may be employed. Ordinary measures, such as the constriction of the bitten limb by a tight ligature above the seat of injury, pressure to squeeze out any poison lying free in the punctures, and stimulating remedies, must not be omitted.

CONCLUSIONS

1. Calmette's antivenene should be used promptly in all cases of snake-bite. It protects effectually against cobra-bites. It probably has little effect against the venom of snakes belonging to other genera; but this matter is still

under investigation, and in any case it is difficult, if not impossible, to be certain of the kind of snake which has inflicted the bite in countries where several kinds are met with. The dose should be 100 cc. or more, if possible.

2. Fuller investigation is necessary with regard to the manufacture of a polyvalent serum applicable to the bites of more than one kind of poisonous snake.

CHAPTER VII

SMALL-POX AND VACCINIA

Causal agent.—That small-pox is due to some living agent similar in nature to the organisms of other infective diseases there can be no doubt, but the actual germ has not been certainly isolated. The most probable parasite is that named by Guarnieri the *Cytoryctes variolæ*, which may be identical with that described by Councilman and by Calkins.

The parasites described by these authors are said to pursue a double life-cycle within the cells, one phase being extranuclear, the other intranuclear. The latter is supposed to correspond with the sexual cycle of the malarial parasite. It is suggested that vaccinia represents the extranuclear phase of the organism, whereas small-pox consists essentially in the invasion and destruction of the nuclei. If these observations are confirmed, the discovery will be a matter of great interest. We shall have an instance of the attenuation of a protozoan parasite taking place by passage through another animal, just as occurs in the case of vegetable parasites (bacteria). Further, a vaccine will have been prepared against a protozoön[1] as well as against bacteria, showing that the human body has the power of forming protective substances against this order of pathogenic agents, as well as against vegetable organisms. Again, the suggestion of an organism undergoing two different cycles within the same animal host, but

[1] Ledoux-Lebard (*Comptes Rendues de l'Académie des Sciences*, 1902, cxxxv., 298) states that he has prepared a specific antiserum to the protozoan organism, *Paramæcium caudatum*, which is pathogenic to some of the lower animals (rabbits and guinea-pigs).

in different positions (cell and nucleus), is of considerable interest.

Identity of small-pox and cow-pox.—The question of the identity or difference of small-pox and cow-pox was long disputed, but there can now be little doubt that Jenner was right in holding that the latter is only small-pox modified by transmission to a different animal, which is less susceptible to the disease.[1] Many attempts have been made to transmit small-pox directly to cattle, and a certain number of successful results have now been recorded. Adult cows take the disease with difficulty; calves are more easily infected. A condition in horses analogous to vaccinia (equine variola) appears to be really the same disease, and to be capable of effecting vaccination.

Complications of small-pox.—Most of the complications arising in small-pox can be distinctly traced to intercurrent infection with pyogenic micrococci (streptococci or staphylococci). It has even been said that the pustular stage of the lesions, which has been regarded as so characteristic of small-pox, as opposed to chicken-pox, can be almost entirely prevented by careful and thorough antiseptic treatment of the skin. If this be true, it would seem that the cutaneous manifestation of the disease is essentially a vesicular eruption, and only accidentally becomes pustular. Abscesses are the commonest complication met with; erysipelas and cellulitis are by no means rare. The ocular affections (keratitis and conjunctivitis) may possibly be due to the virus of the original disease, but here again the action of secondary infections can hardly be excluded Pneumonia, pleurisy, and empyema are also probably instances of intercurrent infection.

VACCINATION

Theory of vaccination.—As already explained, vaccination consists in inoculation of an attenuated form of

[1] *See* Blaxall: *Thirty-first Ann. Report of the Med. Off. of the Local Gov. Board*, 1901–2. Appendix C., i. 568.

small-pox germs, the diminution in virulence being brought about by passage through the body of a calf, a less susceptible animal than man. The attenuated germs are present in the lymph of the vesicles formed on the vaccinated person, and this lymph may be used for inoculation of other individuals, as the germs do not regain their virulence by repassage through man. Vaccinia remains a localised disease, the attenuated germs remaining in the place of inoculation, and not becoming generalised by the blood-stream. At the point of inoculation they form their toxines, which are conveyed all over the body, and stimulate the tissues to form germicidal substances. The cells thus educated retain the property of secreting these substances for a considerable length of time ; in other words, the person vaccinated has gained an active immunity to small-pox and vaccinia.

Preparation of lymph.—It is immaterial, theoretically, from what source, human or bovine, the lymph is derived, but for reasons set forth below, the use of material got from an "animal" source is to be preferred in practice. What is known as "glycerinated calf-lymph" is now chiefly used in this country.[1] This is prepared in the following manner : A supply of stock lymph being already available, a calf is taken, and its abdomen is shaved. A series of incisions are made in it of considerable length, and the stock lymph is rubbed into them. By the fifth day large vesicles have developed along the course of the incisions, and are full of clear fluid, which does not yet exhibit any tendency to become pustular. At this stage the vesicles and their contents are scraped off with a sterile sharp spoon, with all aseptic precautions, and the resulting material is collected in suitable bottles. It is next finely broken up, and triturated with four times its weight of glycerine and water (50-per-cent. solution). The thick creamy fluid produced is run into tubes ; and these are kept in a cold, dark place for some weeks. The result of this treatment is to

[1] In India, in addition to glycerinated lymph, mixtures with vaseline and with lanoline are also employed, apparently with good results.

kill off most of the common pyogenic and similar organisms which might do harm if inoculated; but few, if any, specimens of lymph are actually germ-free. The contagium of vaccinia is left apparently uninjured. It is possible that it exists at this stage in the form of spores, which are resistant to the action of the glycerine. After about a month the lymph is tested bacteriologically, to prove it free from the organisms alluded to; and if it is found to be innocuous, it is drawn into capillary tubes, and is ready for use. The lymph thus prepared is a thick, syrupy fluid, which tends to separate to some extent into a clear and an opaque portion. It is probable that the latter is the active part, and care should therefore be taken not to use only the clear portion in vaccinating. "Chloroform" calf-lymph first introduced by Green[1] is also extensively used in England at the present time. This is prepared as follows:

The lymph is first mixed with water for the purpose of this procedure. Air charged with the vapour of chloroform is then made to pass through a series of tubes of vaccine, and the chloroform is subsequently expelled from the tubes by means of a current of air: thus only the proportion of chloroform which the water can hold in solution (1 : 400) can come in contact with the vaccine. This quantity suffices to kill the bacteria (chiefly staphylococci) generally present, but has no ill effect on the vaccine. The addition to this of any traces of liquid chloroform appears, however, to diminish its activity. The advantages claimed for this method are the speed with which sterilisation is effected, so that in cases of emergency large quantities of vaccine can be rapidly rendered fit for use, and the consequent avoidance of any possible deterioration of strength, such as may perhaps occur during the month or more for which ordinary glycerinated lymph has to stand.

Technique of vaccination.—The essential part of the process of vaccination is that the infective material—the lymph—should be introduced through the epidermis, so

[1] *Lancet*, June 20, 1903, p. 1738.

as to be absorbed by the lymphatics and blood-vessels of the corium. The operation itself is carried out in the following manner :—

The **site** usually chosen is the skin of the outer side of the upper arm over the insertion of the tendon of the deltoid muscle ; sometimes, in females, on the outer side of the thigh just below the great trochanter, or well above the knee on the inner aspect of the thigh. The skin at the seat of operation is, if hairy, carefully shaved, and in any case, well washed with soap and water and then thoroughly scrubbed up with ether. After the ether has evaporated, a tube of lymph is opened, and the whole of its contents ejected on to the clean skin by means of a rubber bulb provided with a plug of sterilised cotton-wool ; a sterile lancet or scarifying needle is used to scratch the epidermis *through* the lymph, care being exercised to avoid drawing blood : the lymph is then rubbed into the scratches. The superfluous lymph is collected on the blade of the lancet and transferred to another spot on the skin about an inch distant, and the process is repeated. Three or four "insertions" are usually made, to obviate risk of failure. The several sites of inoculation should not be too close together —preferably an inch or an inch and a half should be left between them—in order that the resulting vesicles may not coalesce. Should they do so, an unduly sore arm may ensue, and a considerable amount of scarring be finally left. The lymph is left for a few moments to soak in, and the remainder is wiped off the skin with sterile cotton-wool. The wounds are then dressed with dry sterile gauze, bandaged on.

Phenomena of vaccination.—When properly performed, with that careful attention to detail that should characterise every bacteriological procedure, whether carried out in the laboratory or on the operating-table of the surgeon, vaccination results in the formation of a small vesicle with a sharply raised edge, filled with clear serum, and surrounded by a narrow red areola, in from five to

eight days. During the next few days the centre of the vesicle sinks, so as to form a cup-shaped depression, and the fluid contents of the surrounding portion of the vesicle become white and opaque. By the tenth to the twelfth day the vesicle has become a dry scab, which finally separates and leaves a circular depressed scar—"foveated."

About the third day a marked constitutional disturbance is noted—rise of temperature, headache and general malaise, and occasionally sickness and diarrhœa, usually accompanied by some itching at the seat of inoculation. These symptoms generally increase until the vesicle is well developed (eight to ten days), after which they gradually subside. The neighbouring lymphatic glands are enlarged and tender from about the fifth to the tenth or twelfth day. The "beautiful arm" so commonly seen after vaccination in the past century, with its vesicles rapidly becoming pustular, and the accompanying extensive and severe cellulitis often reaching to the wrist and hand, was no criterion of efficient vaccination: it merely formed a pointed commentary upon the contaminated lymph and septic methods of that age.

Revaccination.—In those who have already been once or more vaccinated the phenomena are similar, but less marked. Only a papule may appear, or a poorly developed vesicle with subsequent scabbing. Itching may be the most marked feature. Not very infrequently in such persons revaccination fails entirely.

Number of insertions.—Statistics prove that the protection afforded by vaccination is to some extent proportional to the number of spots at which the lymph is inserted, two "scars" protecting better than one, three than two, and four than three. The practice of making only one insertion is to be condemned as inefficient and conveying a false security. The scars resulting from the vaccination should together make up an area of not less than half a square inch. The table on page 142 shows the nature of the statistical evidence upon which these statements are founded.

TABLE SHOWING THE AGE-INCIDENCE IN VACCINATED CASES CLASSIFIED ACCORDING TO THE CHARACTER OF THE SCARS; EACH CLASS IS REPRESENTED AS COMPRISING A TOTAL OF 1,000 CASES (SANDILANDS.[1])

Character of Scar.	Under 10 years	10-	20-	30-	40-	50 and upwards.
Large (A 1)	15	187	411	253	98	36
Medium (A 2) ...	22	109	248	268	222	131
Small (A 3)	45	112	199	241	211	192
Four or more ...	23	217	441	207	77	35
Three	11	148	350	309	127	55
Two	15	114	273	292	190	116
One	31	129	270	236	203	131
Half or more than half foveated ...	21	188	418	246	99	28
Less than half foveated	15	183	387	263	107	45
Plain scars	27	127	293	238	182	133
Scars absent... ...	83	204	210	157	127	219

Dr. Sandilands points out that "the figures in this table demonstrate a point of some importance—that the incidence in later life is very much greater in the classes with inferior vaccination scars." This point is well brought out in the last column, where it will be seen that those exhibiting the depressed scar typical of a successful vaccination have a case-incidence of less than 3 per cent., whilst among those showing the plain scar resulting from infection with pyogenic organisms only the incidence is five times as great.

It seems at first sight rather difficult to understand the reason for the relation of the amount of protection afforded to the area of vesicles resulting, since it might be supposed that vaccinia being an infective disease, the virus would multiply in the body in any case to an extent only limited by the resistance of the individual, and that therefore one insertion would be as effective in producing immunity as

[1] "An Analysis of the Vaccination Statistics of the Metropolitan Asylums Board for 1901 and 1902." *Lancet*, 1903, ii. 378.

many.[1] The facts being as stated, it seems that the infective organism, whatever its nature, remains localised, in the majority of instances at any rate, within the tissues near the site of inoculation, multiplying to some extent therein, and producing poisons which are carried throughout the system. It would thus bear a close resemblance to the bacilli of diphtheria and tetanus in its mode of behaviour. It is requisite that a certain amount of the poison should be manufactured, in order to cause a sufficient action on the cells of the body to stimulate the formation of the necessary amount of protective substances. Hence the need for a considerable quantity of the virus to be inoculated, the size of the dose being gauged by the number of scars, since possibly the organisms tend to die out somewhat rapidly, being *ex hypothesi* of an attenuated kind.

It is well to remember that the lymph remaining on the arm or other part vaccinated may be *conveyed accidently* to other regions of the body, and that if there is any excoriation at the point of contact, a vaccination-lesion will result. Should such an occurrence take place on the face, a somewhat alarming condition is often produced, the affected part swelling markedly and the neighbouring glands enlarging to a considerable size.[2] The condition is in no way dangerous, but an unsightly scar may be left. A generalised eruption is sometimes produced by such accidental inoculation if it occur in several places.

Nobl[3] has made trial of *subcutaneous injection* of vaccine-lymph in doses of 0·1 to 0·2 cc., diluted with normal saline solution. He finds that there is less local reaction, and no

[1] It seems not impossible that a fallacy of observation may lurk in the inference drawn from the statistics. It is at least conceivable that it is not so much the number of insertions of the lymph that protects, as the careful performance of the act of vaccination, of which the number of scars is some criterion.

[2] The picture thus presented has on more than one occasion been regarded as due to infection by *B. anthracis*, and heroic measures of treatment have been adopted.

[3] *Wien. klin. Wooh.*, Aug. 9, 1906.

greater constitutional disturbance than with the ordinary method. Immunity is rather later in appearing (tenth day). Nobl believes that the method will prove useful in that it avoids risk of local infection and of auto-inoculation; the dose, too, can be exactly measured, and no scars result.

Risks of vaccination.—In the days when it was the practice to vaccinate one child from another by the "arm-to-arm" method, there was a certain element of risk lest some disease should be transferred from one to the other at the same time. Thus it can hardly be denied that *syphilis* has been conveyed in this manner; it seems definitely established that the clear lymph of a vesicle may convey the infection, even apart from contamination with blood. This risk no longer exists when calf-lymph is used.

Secondary infection may take place at the site of a vaccination-puncture, as it may by any other abrasion of the skin. Thus, in a certain number of cases, *erysipelas* has supervened, owing to the subsequent entry of streptococci derived from the insanitary surroundings of the child. It is said that the vaccination in such cases is generally unsuccessful. Milder *septic infection* (probably with staphylococci) may result in a sore arm of unusual severity, and even given rise to glandular abscesses.

In countries where *tetanus* is common, this complication has followed vaccination. MacFarland,[1] from a study of ninety-five such cases, concludes that, although it is after vaccination that the infection with tetanus most often takes place, in some cases the actual lymph may have been contaminated from hay, manure, &c.

The constitutional disturbance produced by vaccination may in some cases be prolonged, taking the form of somewhat severe *anæmia*. Bellotti,[2] who calls attention to this possible sequel, states that children who have been previously rosy and healthy in appearance most often

[1] *Journ. of Med. Research*, May, 1902.
[2] *Gaz. degli Ospedali*, May 10, 1903.

exhibit this condition. He suggests that the organisms of vaccinia may in these rare cases exert a special hæmolytic action.

The names *vaccinia hæmorrhagica* and *vaccinia gangrænosa* have been applied to conditions in which symptoms of unusual severity attend vaccination. In the former a generalised hæmorrhagic eruption develops, which may be accompanied by bleeding from mucous surfaces; in the latter the local lesions, instead of healing, extend deeply and widely, causing necrosis of the tissues and large areas of ulceration, together with severe constitutional disturbance.

It is probable that these conditions are both dependent in the first place upon a debilitated condition of the child, produced by ill feeding, rickets, or tuberculosis; and in the second place upon an invasion by other organisms, such as pyogenic cocci, which either produce local gangrene in the weakened tissues, or give rise to a general septicæmic condition, with hæmorrhagic symptoms. Of the close connection between hæmorrhagic eruptions and general septicæmic states there can be no doubt whatever.

A *keloid* condition sometimes results from the scarring produced in vaccination. This probably has nothing to do with the virus employed, but depends upon a constitutional peculiarity of the individual, in whom any slight traumatism may give rise to a chronic inflammatory overproduction of scar-tissue.

In the absence of an epidemic of small-pox a child should not be vaccinated when it is obviously in bad health. Not only will the parents attribute to the operation any increase in the symptoms of the existing condition which may ensue, however accidentally, so that the procedure will incur some degree of disrepute with them and with their ignorant neighbours, which it is better to avoid, but it is probable that in some instances the constitutional disturbance produced by the inoculation may unduly depress a child already weakened by existing disease. Children

suffering from eczema, herpes, or other skin-diseases should not be vaccinated, if the matter is not urgent. Generalisation of the vaccinial eruption is said to occur in such patients, but the evidence is not very clear. Hæmophilic subjects should not be vaccinated, the risk to them being greater from any source of bleeding than from the continued liability to small-pox.

Insusceptibility to vaccination.—It is said that some persons are by nature insusceptible to vaccination. This may possibly be the case occasionally, but instances of such a condition which will stand investigation must be very rare indeed. Thus, Thorne[1] states that 107,180 vaccinations have been done by public vaccinators under the Local Government Board without one instance of failure. Cory[2] reports one case, among 38,000, in which he was twice unsuccessful in attempting to vaccinate an infant. Bryce[3] records ninety-eight unsuccessful attempts to vaccinate with calf-lymph out of 126,000 vaccinations. It is not unusual, in attempting to revaccinate an adult, to find it impossible to produce any effect recognisable as vaccinia. The same is of course true, and to a still greater degree, of those who have suffered from small-pox.

It is necessary to make three attempts at (primary) vaccination before pronouncing any individual insusceptible.

Supply of lymph.—In the present state of the law in this country, public vaccinators are supplied by the Local Government Board with lymph which is prepared under careful State supervision. This lymph is not to be obtained by other practitioners, who are dependent for their material upon the lymph offered in the market by private trading establishments. No supervision of any kind is exercised over these manufactories, so that only the pressure

[1] *Twenty-seventh Annual Report of the Med. Off. of the Local Government Board*, p. viii.

[2] "Lectures on Vaccination," p. 73.

[3] *Boston Med. and Surg. Journ.*, Feb. 26, 1903.

of competition with other firms, and the risk of losing custom if their product is found inert, are to be relied upon to ensure the purity and efficacy of these lymphs. Such a state of things appears entirely indefensible. It is much to be hoped that in future Acts of Parliament dealing with vaccination, provision will be made for the inspection of private vaccine establishments, and for the testing by State officials of all lymph put upon the market.

Protection afforded by vaccination.—Of the value of the protection afforded by vaccination against small-pox there can be no doubt in the mind of anyone who is willing to look facts in the face and draw conclusions without pre-existing bias. Before Jenner introduced his great discovery to the world the disease was universally prevalent. It was regarded as a children's disease, owing to the fact that all were susceptible and contracted small-pox at the earliest opportunity. It thus caused an immense infantile mortality; but it also attacked adults of all ages and all positions in life. Princes were no more sacred from its attack than the poor; scarred faces were the rule rather than the exception. Hence the new prophylactic was welcomed with delight throughout the world, and special measures were taken to introduce it and to carry a supply of lymph into the most distant countries.

At the present day, owing to the general practice of vaccination, small-pox is a rare disease, and its very rarity has caused a certain degree of carelessness in carrying out the prophylactic procedure. Hence there are signs that in this country the disease is making attempts to re-assert itself; and places, such as Gloucester and Leicester, where the fanatical opponents of vaccination have gained the ascendency and succeeded in causing general neglect of the precaution, have paid the penalty for their folly by suffering from severe epidemics.

The general recognition of the value of vaccination is shown in the regulations adopted by most life-insurance offices, which charge an additional premium to all those who

have not been vaccinated. In view of the general protection of the community, the risk is small and the addition slight; but there can be no doubt that, if small-pox once more became prevalent, this additional percentage would be considerably increased. Vaccination, or revaccination, is also compulsory upon recruits for the army and navy, and upon those employed in the postal service.

The statistics of the German army and of the civil population[1] in that country afford convincing evidence of the benefits derived from vaccination, if any be still needed. Directly vaccination was introduced into the army the average deaths per 100,000 (taking an average of the 10 years before and the 10 years after its initiation) fell from 36 to 3, whereas in the civil population the relative numbers were 26·9 and 19·4 respectively, showing no such tendency to fall. At the same time the existence of small-pox among the civil population was a source of infection even to the protected members of the army, a small number of cases continuing to occur. It was only after vaccination was enforced universally that the disease practically disappeared from the army, while among the civil population it at once fell almost to vanishing point.

We may prove the value of the protection thus afforded by vaccination, by means of a comparison of the German army with others not so protected. In the German army, from 1875 to 1887, only 148 cases of small-pox occurred, whereas in the Austrian army, not protected by systematic vaccination, there were 10,238 cases between 1873 and 1886, and in the French army, from 1875 to 1881, 5,605 attacks.[2] In Sweden, in pre-vaccination days, 2,050 deaths occurred annually from variola; after its introduction the average mortality fell to 169 per annum. In Bohemia, with

[1] See Statistical Chart quoted in Marx, "Die Experimental Diagnostic Serumtherapie u. Prophylaxe der Infectionskrank.," 1907.

[2] Immermann, art. "Vaccination" in Nothnagel's "Encyl. of Pract. Med.," English ed., 1902.

a population of 3,039,722, the average annual deaths for small-pox were 7,663; after vaccination was introduced they fell to 282, though the population had risen meanwhile to 4,248,155. Thus the small-pox mortality fell, owing to vaccination, from 1 in 397 of the population to 1 in 14,741 —a sufficiently striking decrease.

There is no doubt that Jenner was wrong in considering that vaccination once performed conferred an absolute immunity against small-pox; and failure to recognise certain limitations in this respect has done harm by enabling disbelievers in the practice to create a distrust in the minds of the ignorant by pointing to individual instances of failure, where complete protection had been promised. That a person who has been once vaccinated may afterwards suffer from small-pox is undoubted, although it is almost always the case that the subsequent attack of the disease is relatively mild (modified small-pox). A certain number of deaths do, however, occur even among those who have been vaccinated. Even revaccination does not necessarily confer absolute immunity.

In the first place, there is now no doubt that in many persons the period of immunity after vaccination is not indefinitely prolonged. Perhaps seven years may be taken as the average period of fairly complete protection, but probably even during this time the degree of resistance is constantly diminishing. In the second place, it is most probable that modifications of general health may affect the individual's resistance to this, as to other diseases, even when immunity has been produced. Fatigue or ill health may perhaps temporarily reduce the powers of defence. The longer, therefore, the period which has elapsed after vaccination, the less the degree of protection that is likely to persist, and the more easily will depressing circumstances suffice to reduce it below the point necessary to confer immunity. The following table shows the gradually diminishing protection afforded by

vaccination, and the consequent increase of mortality as age advances :—

TABLE SHOWING THE PERCENTAGE MORTALITY AT SEVERAL AGE-PERIODS AMONG THE SAME SCAR-BEARING VACCINATED CASES AS ARE SHOWN IN THE FORMER TABLE, p. 142 (SANDILAND)[1]

	Under 10 years.	10-	20-	30-	40-	50-	60-	70 and upwards.
Mortality	3	2	7	5	22	21	24	20

Hence it cannot be too strongly insisted upon that not only vaccination, but *revaccination*, is needful to protect the individual and society against small-pox. Children should not only be vaccinated soon after birth—within the first three months of life—but revaccinated perhaps on going to school, and certainly on leaving it. Should small-pox be at all prevalent, adults will be wise to have the operation repeated, if more than seven years have elapsed since they last underwent it. If as a matter of fact they are still immune, the vaccination will not "take," and they will suffer no inconvenience; while if it succeed, they will have the satisfaction of knowing that they have gained a new lease of immunity.

In this connection we may quote the following remarks by Dr. Sandiland[2] with regard to the protection of the community at large by vaccination :—

"It cannot be too much emphasised that the extraordinary diminution in the mortality from small-pox in the last century has been due, not so much to the protection of a majority of the population, as to the absolute immunity of a minority, probably made up from persons at all periods of life, who are continually standing in the way of small-pox infection, and compelling it to travel by long and circuitous routes before alighting, scattered and diluted, on patches of soil in which it can take root and flourish.

[1] *Op. supra cit.*

[2] *St. Bartholomew's Hosp. Journ.*, July, 1903, p. 155.

"Again, a person saved from small-pox by vaccination should not, so to speak, be counted as one, but rather should be represented by a figure standing for himself and all those whom he would have infected had he been overtaken by the disease. It is this process of the multiplication of the benefits of vaccination which has reduced the small-pox mortality in England out of all proportion to the protective power of infantile vaccination, and which makes it reasonable to anticipate with confidence that if revaccination in adolescence were added to infantile vaccination, small-pox would disappear, as indeed it has disappeared in Germany."

Modified small-pox.—Small-pox occurring in persons who have been vaccinated is generally of the kind known as "modified" small-pox. The eruption is generally scanty and comes out rapidly, becoming vesicular within 12 to 24 hours. Some of the papules may never develop into vesicles. The vesicles which do form are often smaller than those seen in the unmodified disease, and many of them dry up without becoming pustular. The crusts fall off more rapidly than in ordinary small-pox, and less pitting is generally left behind. The constitutional symptoms are much less pronounced, and often subside entirely within two or three days, the patient being practically well within a fortnight of the onset. Complications are infrequent and scarcely ever severe.

Rapidity of protection gained.—With regard to the exact period at which immunity to small-pox is produced by vaccination—*i.e.* on which day after the performance of the inoculation—it is difficult to be certain. No doubt the immunity is a gradually increasing one, but it is probably slight before the vesicles are well developed, and is mainly brought about from this period to the time when they become purulent. According to Bryce,[1] protection is complete by the fourth day after vaccination, and only a modified small-pox is likely to ensue in cases in which exposure to infection is contemporaneous with vaccination, a fatal issue being improbable in such a case. It will be remembered that the incubation-period of small-pox is

[1] *Boston Med. and Surg. Journ.*, Feb. 26, 1903.

usually about twelve days, so that vaccinia will have time to develop to its full extent in the interval between infection and the onset of symptoms. According to E. Hart,[1] immunity reaches its maximum about the fourth week after vaccination. There is little doubt that individuals vary as to the rapidity with which protection is gained, as well as with regard to the length of time for which it remains. The "memory" of tissue-cells with regard to the production of immunising substances is as liable to vary as the mental memory for events.

SERUM-TREATMENT

Serum of immune cattle.—Thomson and Brownlee[2] made experiments with regard to a possible antitoxic influence, upon patients suffering from small-pox, of the serum derived from heifers which were immune to vaccinia. Large quantities of the serum were injected, but the results were apparently quite negative. In certain cases a modified form of the disease occurred; but, as the patients had been vaccinated, it was probably to be attributed to this cause. The serum did not appear to influence the course of vaccination (revaccination) in one case.

Antistreptococcic serum.—With a view to diminishing complications, the use of antistreptococcic serum has been suggested (Lindsey). Schoull[3] has made a practice of injecting 60 cc. of this serum in doses of 20 cc., and gives even more than this in severe cases. No pain or reaction is induced by the injection, which is given in the flank, all antiseptic precautions being taken. He claims that rapid improvement results in all the symptoms which are connected with the eruption. The painful condition of the face subsides: photophobia, dysphagia, and hoarseness diminish; pruritus is checked. In some instances a single

[1] Allbutt's "System of Medicine," 1897, ii. 578.

[2] *Lancet*, April 4, 1903.

[3] *La Semaine Méd.*, March 11, 1903; *Med. News*, April 25, 1903, p. 794.

injection of the antistreptococcic serum produced an immediate fall of temperature. Even hæmorrhagic cases may recover under this treatment. In all Schoull treated five hæmorrhagic, eight confluent, and nine discrete cases. Out of these, two patients died (9 per cent.), whereas the general mortality in cases not so treated was 20·5 per cent. Alfred Smith [1] speaks enthusiastically of this method of treatment, as shortening the duration of the disease and preventing pitting and complications. The serum should be used early, and in sufficient quantities (20 cc., repeated).

CONCLUSIONS

1. Vaccination confers an *active immunity* against small-pox, and protects almost absolutely for a certain period of time. This immunity gradually diminishes, and in many cases disappears after a longer or shorter interval, which varies in different individuals. The immunity may be renewed by revaccination.

2. In a person who has been even once vaccinated small-pox generally occurs, if at all, in a modified form, which is comparatively seldom fatal.

3. If calf-lymph, duly sterilised, be used, the danger of any ill effects resulting is very small indeed. Complications are generally due to want of cleanliness and lack of care in the after-treatment of the lesions resulting from the inoculation.

4. Complications in the course of small-pox are generally due to intercurrent infection with pyogenic organisms, and there is reason to believe that the use of antistreptococcic serum may prove beneficial in averting or modifying them.

5. Attempts to treat the disease with a serum derived from immune cattle (*anti-microbial?*) have been unsuccessful.

6. No *antitoxic* serum is known.

[1] *Med. Record*, April 2, 1904, p. 533.

CHAPTER VIII

HYDROPHOBIA (RABIES)

Causation.—Up to the present time the actual cause of hydrophobia, or rabies as it is called when it affects the lower animals, is absolutely unknown. A large number of organisms have at one time and another been announced as the excitants of the disease (bacteria, protozoa, &c.), but no one of them has so far withstood the test when its claims were more fully investigated.

Most recently of all, Negri[1] has described parasites in the large nerve-cells of the cerebral cortex, cerebellum, &c. Some of the organisms contain a number of small refringent bodies like spores. They appeared as a rule just before the onset of symptoms in the rabbit, and were found in one case of human hydrophobia. Negri regards the bodies as protozoa, and states that they occur only in hydrophobia and not in other conditions. They are easily demonstrable by ordinary staining methods. Remlinger and Riffat Bey[2] state that they have succeeded in passing the virus of rabies through a Berkefeld filter; if this be confirmed, it is necessary to conclude that the infective agent is capable of existing in a very minute form, at one period, at all events, of its life-cycle.

The virus or infective material, whatever its nature, resides in the saliva of infected animals, as is evident from the fact that the disease is most often conveyed by bites of rabid animals; but it exists in still greater concentration in the central nervous system (brain and spinal cord). It

[1] *Zeitschr. f. Hygiene u. Infectionskrank.*, 1903, xliv. 507.

[2] *Comptes rendues de la Soc. de Biol.*, 1903, lv. 730.

is probably not present in the blood or in most of the organs of an animal which has died from the disease, but is found in the secretions of certain glands (lachrymal, mammary, pancreatic); possibly the poison is excreted by these channels. The toxine of rabies bears very close resemblance to that of tetanus in many of its properties. Thus it has a marked affinity for the nervous system, passing to the central portions of this by way of the peripheral nerves (*cf.* p. 109); it produces first a stimulation of the reflex activity of the nervous centres, though this is followed later on by paralysis; its effects on the cerebrum are manifested by excitement and delirium analogous to the phenomena noted as the result of injections of tetanine into the brain-substance. Further, the occurrences in the wound itself bear some resemblance to those met with in tetanus, as in each case the injured point may cicatrise, but with the onset of the disease pain may occur in the scar; while Pace[1] has shown that the virus of rabies remains locally at the seat of inoculation, as do the bacilli of tetanus. Deep wounds of a lacerated nature are those most liable to give rise to hydrophobia, just as injuries of this sort are those most commonly followed by tetanus.

Statistics as to the percentage number of all cases bitten by rabid animals which subsequently develop hydrophobia are somewhat difficult to obtain. Rose Bradford[2] puts it at 16 to 25 per cent.; some authorities give rather higher, others lower, figures. It is, at any rate, certain that all who are bitten do not develop the disease, even apart from treatment. This fact is of importance in estimating the benefits derived from preventive inoculations. When once it has appeared, the disease is invariably fatal. Persons bitten through their clothes are not very likely to be attacked by hydrophobia, as the virus is wiped off the teeth of the animal in passing through the dress-material.

[1] *Ann. de l'Inst. Pasteur*, 1903, xvii. 293.

[2] Art. "Hydrophobia" in Quain's *Dictionary of Medicine*, 3rd edition, by Montague Murray. 1902.

Natives of India and other hot countries are thus more liable to suffer from hydrophobia than are Europeans resident in the same districts, owing to the scantiness of their clothing.

Incubation-period.—The incubation-period of hydrophobia is very long, varying from about three weeks to (possibly) some years. As to the extremely long periods assigned to the incubation of this disease, there is considerable doubt. Kaspareck and Teuner [1] relate a case in which the disease occurred seven months after infection, in spite of prophylactic inoculation. Pampoukis,[2] out of a number of cases not treated in any way, found that 9·3 per cent. occurred within the first month after the bite, 53·4 per cent. in the second month, and 37·2 in the third month. Probably six weeks may be looked on as the average period of time between the injury and the onset of symptoms.

Modification of the virus of rabies.—Although nothing is known of the poisonous material which gives rise to this malady, yet experiments show that it resides chiefly in the nervous system of infected animals, and that it can be modified in various ways. Thus, light, air, and desiccation rapidly destroy the virulence of rabic matter. Heat, also, has the same effect, and so has the addition of antiseptic drugs, though the resistance offered to these last is considerable. Carbolic acid (1 : 20) cannot be relied upon to destroy the virulence of emulsions of brain-substance in less than an hour, and perchloride of mercury (1 : 1,000) takes three hours to sterilise this fluid. Digestion with gastric juice diminishes the virulence of infected spinal cords; and this method of producing a vaccine has been employed in Italy, and is known as the "Italian method." Post-mortem decomposition has little effect in destroying the virus of rabies, which may remain potent for at least a month after burial of a carcase. Glycerine is a good preservative of the virus.

[1] *Berlin. klin. Woch.*, 1902, Sept. 8, p. 844.
[2] *Ann. de l'Inst. Pasteur*, 1900, p. 111.

Exaltation of virulence may be effected by passing the virus through a succession of rabbits, which are very sensitive to the disease. After passage through a large number of these animals the incubation-period is gradually shortened from about three weeks or a little less to a constant period of six or seven days. Virus of this degree of virulence is called by Pasteur "virus fixe," [1] and is used in the preparation of his vaccine. There is reason to believe that the virus which has thus been exalted in virulence for rabbits is really attenuated for mankind. Thus Nitsch [2] inoculated himself with fresh cord from a rabid rabbit with no ill effects.

ANTIRABIC VACCINATION

Pasteur's vaccine.—Pasteur discovered that by drying the spinal cords derived from rabid animals for varying periods of time he could prepare a series of viruses of graduated strengths. Thus, if such a cord is dried for fourteen days, it loses all its toxic potency; if it is submitted to this process for only three or four days, the virulence is but little reduced. Immunity to rabies, as to other infective diseases, can be induced by injecting at first minute doses of the organism or toxine, and gradually increasing the doses until quite strong virus can be employed. Graduation of the dose is effected by taking equal amounts of nervous matter from spinal cords which have been dried for varying lengths of time. The actual vaccine consists of a small quantity (2–3 mm. length) of the substance of the spinal cord of a rabbit which has been killed by inoculation with the "fixed virus," rubbed up into an emulsion with 5 cc. of sterile broth or salt-solution. About 3 cc. of the resulting fluid are injected. A cord dried for fourteen days is used for the first injection: on succeeding occasions emulsions of less attenuated virus are used, till finally a portion

[1] As opposed to the virus of uncertain strength (*virus de la rue; Strassenwuth*) derived from accidentally infected animals.

[2] *Wien. klin. Woch.*, 1904, No. 36, p. 963.

of a spinal cord dried for only three or four days is employed. A scheme of the actual doses may be thus drawn up :—

Ordinary Treatment.		Intensive Treatment.	
	Cord dried.		Cord dried.
First day—Morning	14 days	2 injections	14 and 13 days
Evening	13 „	„	12 and 11 „
Second „ Morning	12 „	„	10 and 9 „
Evening	11 „	„	8 and 7 „
Third „ Morning	10 „	1 injection	6 days
Evening	9 „	„	„
Fourth „ Morning	8 „	„	5 days
Evening	7 „	„	„
Fifth „ Morning	6 „	„	„
Evening	6 „	„	„
Sixth „ Morning	5 „	„	4 days
Seventh „ „	5 „	„	3 days
Eighth „ „	4 „	„	4 days
Ninth „ „	3 „	„	3 days
Tenth „ „	5 „	„	5 days
Eleventh day „	5 „	„	„
Twelfth „ „	4 „	„	4 days
Thirteenth „ „	4 „	„	„
Fourteenth „ „	3 „	„	3 days
Fifteenth „ „	3 „	„	„
		On the following 6 days 6 more injections of 5-, 4-, 3-, 5-, 4-, 3-day cords respectively.	

A more rapid form of vaccination is used in cases in which the bites are about the face and head, as in these cases the incubation-period is usually shorter, and therefore it is important to produce a full degree of immunity as quickly as possible. This is known as "intensive" treatment. It will be seen in the scheme given that the virulent matter contained in a cord only dried for three days is here administered on the seventh day, instead of on the ninth, as in the ordinary method.

The exact arrangement of the doses varies a little at different institutions. Marx states that in Berlin it is considered that the virulence of the dried cord is lost about the eighth day, instead of the fourteenth. Hence the Berlin

authorities consider that the first few days of the Paris treatment are wasted, only material which is quite inert being inoculated; they, therefore, adopt a scheme according to which on the first day cords dried for seven and eight days are administered; on the second day, cord of six days' drying, and so on, reaching a cord dried for three days on the sixth day of treatment. Then cords of five, four, and three days' drying respectively are each administered for two days, and on the fourteenth and fifteenth days cords only dried for two days. Then for the last four days of the treatment slightly less virulent material is again employed. In the intensive treatment at Berlin a cord of three days' drying is reached on the evening of the third day of treatment, and one of two days' on the eighth day. The whole intensive course, here also, lasts twenty-one days.

Nitsch [1] recommends starting with cord dried for six days, and giving two injections daily, reaching material that has been dried for only one day on the tenth (last) day of treatment. He uses also larger quantities of the vaccine (3–10 mm. of the cord) in emulsion.

Institutes for antirabic inoculation are now numerous. Besides the Paris "Pasteur Institute," there exist institutes at Lille, Marseilles, Montpellier, Lyons, and Bordeaux, in France; at Berlin, Vienna, Buda-Pesth, Berne, Odessa, Constantinople, Algiers, Kasauli (India), &c. Different modes of preparing a virus of diminished virulence for purposes of inoculation are adopted in different countries. Thus, the Italian method of Tizzoni and Centanni is to treat the spinal cords with gastric juice, which has an attenuating effect on the virus. Hogyes, in Buda-Pesth, merely dilutes an emulsion of virulent material to different degrees, using a high dilution for the first injections, and gradually raising the strength on succeeding days. The theory underlying this procedure is that the usual method of attenuation by drying alters the quantity of the virus, but not its quality; in other words, it kills a certain proportion of the germs

[1] *Wien. klin. Woch.*, 1904, No. 36, p. 959.

present, so that a smaller number of them are injected at a dose, but it does not alter their virulence. Hence, the same result may be obtained by simple dilution. The practical results of this method seem to bear out the theory on which it is founded, as very favourable statistics of the work of Hogyes' institute are shown.

Effects of antirabic vaccination.—The effect of Pasteur's method of vaccination in cases of bites by rabid animals is to produce an active immunity. Since the infective agent in rabies is not known, it is impossible to say with certainty whether the immunity depends on an antitoxine or on a germicidal state of the serum and tissues. The latter is the more probable, as it has already been shown that the virus must contain a living organism, not merely a toxine. Owing to the long incubation-period of hydrophobia it is possible to induce immunity to the disease between the time at which the bite was inflicted and that at which the symptoms commence. Thus the treatment is in reality prophylactic, and not in any way curative. If the symptoms have already set in, Pasteur's treatment is of no avail. The analogy to ordinary vaccination (against small-pox) is exact. In the latter, vaccination carried out at the time of exposure to infection may protect against the disease, since the incubation-period of vaccinia is shorter than that of small-pox. The difference here, however, is not very great, and more often such vaccination will only lessen the severity of the ensuing attack of small pox. In the case of rabies, which has an incubation period of about six weeks as a rule, there is full time for immunity to be produced before the disease appears, and protection is thus usually complete.

Results of the treatment.—A good deal of scepticism was expressed as to the value of Pasteur's treatment when it was first introduced; it was even suggested that it might result in conveying the disease instead of preventing it, and it is possible that accidents of this kind have actually occurred. At the present time there can no longer be any

doubt as to its efficacy, or as to the boon conferred on the human race by its discovery. The exact mortality from hydrophobia in all cases of bites by rabid animals, in times before the inoculation-treatment was introduced, cannot be exactly calculated, but it may safely be put at not less than 10 per cent., whereas now among the cases treated at the various Pasteur Institutes the death-rate has been reduced to a fraction of 1 per cent. The following table shows the annual mortality at two separate institutions—the original Pasteur Institute in Paris[1] and the similar foundation in New York.[2]

TABLE SHOWING DEATHS FROM HYDROPHOBIA AMONG CASES TREATED IN PARIS AND NEW YORK

Year.	PARIS.			NEW YORK.		
	No. of Cases.	Deaths.	Per-centage.	No. of Cases.	Deaths.	Per-centage.
1886	2,671	25	0·94	—	—	—
1887	1,770	14	0·79	—	—	—
1888	1,622	9	0·55	—	—	—
1889	1,830	7	0·38	—	—	—
1890	1,540	5	0·32	160	0	0
1891	1,559	4	0·25	100	2	2
1892	1,790	4	0·22	104	0	0
1893	1,648	6	0·36	85	0	0
1894	1,387	7	0·50	89	1	1·12
1895	1,520	5	0·33	167	2	1·19
1896	1,308	4	0·30	236	0	0
1897	1,521	6	0·39	133	1	0·74
1898	1,465	3	0·20	125	1	0·8
1899	1,614	4	0·25	159	2	1·2
1900	1,420	4	0·28	241	1	0·43
1901	1,321	5	0·38			
1902	1,105	2	0·18	—	—	—
1903	628	2	0·32	—	—	—
1904	755	3	0·39	—	—	—
1905	727	3	0·41	—	—	—
1906	772	1	0·13	—	—	—
1907	786	3	0·38	—	—	—
1908	524	1	0·19	—	—	—

[1] Viala, *Ann. de l'Inst. Pasteur*, 1909, p. 509.

[2] Rambaud, *Med. News*, 1902, i. 635. We have not been able to find the New York statistics for later years.

It will be seen from these figures that the death-rate has never reached 1 per cent. in Paris since the Institute was started, while in New York the percentage was only thrice over that amount in the years recorded above.

Very full statistics are published by the various Pasteur institutions as to the exact nature of the cases treated, in which these are tabulated according to the region of the bite and the evidence available as to the reality of the disease from which the dog or other animal that inflicted the injury was suffering. In the tables below, Class A contains cases in which the dog was proved by conclusive evidence to be rabid; Class B, those in which rabies was certified by a veterinary surgeon, as a result of examination; and Class C, those in which the nature of the disease in the animal was doubtful. The injuries are classified, as a rule, according as they were on the face, hands, or lower limbs, the last being usually covered with clothes.

TABLE OF CASES TREATED IN THE PASTEUR INSTITUTES OF PARIS (1901) AND NEW YORK (1900–1)

	Bitten on Head.			Bitten on Hands.			Bitten on Lower Limbs.			Total.		
	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.	Treated.	Died.	Mortality.
Class A	20	0	0	93	0	0	58	0	0	171	0	0
	13	**1**	**7·69**	**62**	**0**	**0**	**13**	**0**	**0**	**88**	**1**	**1·13**
Class B	80	0	0	521	4	0·77	184	0	0	785	4	0·51
	7	**0**	**0**	**47**	**0**	**0**	**6**	**0**	**0**	**60**	**0**	**0**
Class C	23	1	4·34	186	0	0	153	0	0	362	1	0·23
	13	**0**	**0**	**53**	**0**	**0**	**27**	**0**	**0**	**93**	**0**	**0**
Total	123	1	0·79	800	4	0·50	395	0	0	1318	5	0·38
	33	**1**	**3·03**	**162**	**0**	**0**	**46**	**0**	**0**	**241**	**1**	**0·4**

In the above table the figures derived from the New York Institute are in dark type.

In the table on page 164 are given the figures supplied by the Indian Pasteur Institute at Kasauli,[1] under Major D. Semple, M.D., R.A.M.C., which are on a slightly different system, the classes being, however, the same. The statistics for Europeans and natives are given separately, the latter being liable to more extensive and dangerous bites owing to their lighter clothing.

In 1905, 1,145 persons were treated, with 7 failures; and in 1906, 1,147 persons, with 9 failures.[2]

Ferré[3] records that at Bordeaux there were treated, in 1901, 100 cases of bites by rabid animals, with no deaths. Trolard,[4] in Algiers, treated 1,836 patients, among whom there occurred 9 deaths (0·49 per cent.). In Tunis,[5] up to the end of 1906, 2,490 cases had been treated, with 9 deaths —a mortality of 0·36 per cent.

Site of injection.—The immunising injections are generally administered subcutaneously over the abdomen, as here it is easy to avoid injury to any nerves. Krasmitski[6] recommends intravenous injection of a filtered emulsion, as producing a more rapid protection. He states that he has successfully treated 70 cases in this manner without any ill effects.

The importance of *early treatment* after the injury has been inflicted is proved by the statistics of the Odessa Institute,[7] which show that of 4,602 cases treated within the first week, 26 deaths occurred, giving a mortality of 0·56 per cent.; among 961 treated in the second week, 16 died, or 1·66 per cent.; while among 313 treated in the third week, 10 deaths ensued, a mortality of 3·19 per cent.

[1] *Annual Report of the Sanitary Commissioner with the Government of India*, 1901, p. 128.

[2] *Ibid.*, 1905 and 1906.

[3] *Ann. de l'Inst. Pasteur*, 1902, p. 391.

[4] *Ibid.*, 1900, xiv. 190.

[5] Nicolle, *Arch. de l'Inst. Pasteur de Tunis*, 1907, i. 35.

[6] *Ibid.*, p. 393.

[7] Quoted by Deutsch and Feistmantel, *Impstoffe und Seru*, Leipzig, 1903.

TABLE SHOWING RESULTS OBTAINED IN THE INDIAN PASTEUR INSTITUTE, 1901

CLASSES.	SUB-CLASS I. BITTEN ON THE HEAD OR FACE.			SUB-CLASS II. BITTEN THROUGH THE EXPOSED SKIN ON ANY PART OF THE BODY OTHER THAN THE HEAD OR FACE.			SUB-CLASS III. BITTEN THROUGH THE CLOTHING.			TOTALS.		
	Treated.	Failures.	Percentage Mortality.	Treated.	Failures.	Percentage Mortality.	Treated.	Failures.	Percentage Mortality.	Treated.	Failures.	Percentage Mortality.
CLASS A—												
Bitten by animals proved rabid	**9**	**0**	**0**	**63**	**0**	**0**	**14**	**0**	**0**	**86**	**0**	**0**
	6	0	0	81	3	3·7	5	0	0	92	3	3·26
CLASS B—												
Bitten by animals certified rabid	**2**	**0**	**0**	**43**	**0**	**0**	**7**	**0**	**0**	**52**	**0**	**0**
	1	0	0	38	0	0	3	0	0	42	0	0
CLASS C—												
Bitten by animals suspected rabid	**7**	**0**	**0**	**47**	**0**	**0**	**23**	**0**	**0**	**77**	**0**	**0**
	15	0	0	155	2	1·29	24	0	0	194	2	1·03
TOTAL	**18**	**0**	**0**	**153**	**0**	**0**	**44**	**0**	**0**	**215**	**0**	**0**
	22	0	0	274	5	1·83	32	0	0	328	5	1·52

In the above table the figures relating to Europeans are in dark type.

SERUM-TREATMENT

Antirabic serum.—The serum of animals immunised by the Pasteurian method is capable of neutralising the virus of the disease. If a sufficient amount of the serum be mixed with an emulsion of virulent spinal cord and injected into a rabbit, no symptoms of disease will develop. As previously mentioned, in the absence of all knowledge of the causal agent of hydrophobia it is impossible to ascertain whether the serum is antitoxic or germicidal; but probabilities are in favour of the latter. Tizzoni and Centanni,[1] as the result of prolonged experiments, suggested the use of this serum as a protective against the disease in persons who had been bitten, instead of the Pasteurian treatment. They consider that their method is quicker and equally certain. In some cases, also, this serum may act as a cure (in rabbits) when the symptoms of the disease are just beginning, a period at which ordinary immunising treatment would be absolutely useless.

Preparation.—The method of preparing the serum is by inoculating sheep with rabic material attenuated by the "Italian method" (p. 159). For the first series of inoculations, seventeen injections in all are given over a period of twenty days, each dose consisting of 0·25 grm. of virus for every kilogramme of body-weight. The injections are given subcutaneously. Later on, immunity is kept up by further inoculations at intervals of two or two-and-a-half months. The serum is withdrawn on the twenty-fifth day after the last injection. The fresh serum may be dried at a gentle heat over sulphuric acid, and preserved in this form indefinitely.

The serum thus prepared will protect animals against rabies when administered in doses equivalent to 1/25,000 of the body-weight. One-and-a-half drops may protect an animal weighing 2 kilogrammes. A serum of this strength is called "typical serum" (S. T.).

Tizzoni and Centanni state that their serum is applicable

[1] *Lancet*, 1895, ii. 659, 727 and 780.

to man, and recommend that doses of 20 cc. should be used, given in three injections—one-half first, then the remaining half in two other doses at intervals of three days. The above amount is advised for cases which come under treatment within the first four days after the bite. For cases seen between the fourth and fifteenth days the amount of serum should be doubled, and very large quantities should be given in cases of bites about the face and head.

Serum-treatment has been carried out at the Kasauli Institute by Lieut.-Colonel Semple in the case of badly-bitten persons and of those who come for treatment some time after being bitten. The serum is prepared from ponies. "A dose of serum is given on the first day, after which the usual methods of treatment are carried out." It is hoped that a passive immunity may thus be induced so as to render the patient safe against infection during the period before the active immunity comes into play.[1] It would be well to inject some of the serum in any case in which pain or discomfort began to be felt in a wound inflicted by a bite, after this has healed up, even before any symptoms of hydrophobia were manifested. The serum is quite harmless in any case.

CONCLUSIONS

1. In all cases of bites by rabid animals, recourse should be had as soon as possible to antirabic inoculation. It is important that this should not be delayed. There is practically no danger in the procedure.

2. If possible, in cases where there is doubt as to whether a dog which has bitten anyone is rabid or not, the animal should *not* be killed at once, but should be kept under close observation. In this way a positive diagnosis can usually be made in less than ten days; otherwise it may be necessary to have recourse to experimental inoculations

[1] See *Ann. Report of the Sanit. Commission with the Gov. of India*, 1902, p. 115; Semple, *Lancet*, 1908, i. 1611.

to decide the question, and such experiments take three to four weeks. It would not be safe to await the results of these experiments before beginning treatment.

3. If for any reason the preventive treatment has been put off till unduly late, it would seem advisable to inject antirabic serum as a prophylactic measure, should it be available.

CHAPTER IX

PLAGUE

Causal organism.—The *Bacillus pestis* was discovered by Yersin in 1894. It does not appear to form virulent poisons in culture-media,[1] but the bodies of the bacteria themselves are highly toxic. By its action as a parasite, the organism produces a "hæmorrhagic septicæmia," that is to say, a general infection (the organisms multiplying in the blood-stream), with interstitial hæmorrhages in the various organs. Curative serums have been prepared for the treatment of the disease, and protective vaccination has been carried out.

HAFFKINE'S PROPHYLACTIC

Preparation of vaccine.—Haffkine prepares his vaccine by growing *B. pestis* in flasks of broth to which a few drops of fat or oil have been added. Each drop, as it floats on the surface of the liquid, acts as a focus for the development of the organisms, which form colonies hanging down into the broth in the shape of stalactites. The vessels are shaken from time to time, by which means the hanging colonies are thrown down into the fluid, and others form in their places. After growth has gone on for a month or six weeks, the bacilli are killed by heating to 70° C. for one to three hours, and the fluid is tested by culture to make certain that it is sterile; after which it is ready for use as vaccine. The usual dose for an adult man is 3 cc., for a woman rather

[1] Klein's experiments, however, seem to show that some toxic material is contained in the fluid of broth-cultures of the bacilli (*see* p. 170).

less (2 to 2½ cc.); children receive still smaller amounts The vaccine is given by subcutaneous injection into the arm. The administration is followed by redness and swelling at the seat of inoculation, and constitutional symptoms in the form of rise of temperature and feeling of illness. The latter pass off in about twenty-four hours, but the patient should spend the first day after the treatment at rest, not resuming his ordinary avocations till the second day.

Results of inoculation.—Haffkine considers that protection against plague is produced rapidly—at the end of twenty-four hours. In view of the facts ascertained by Wright with regard to antityphoid inoculation, it seems likely that there may be at first a period of increased susceptibility to infection, and this has been asserted by Calmette. Bannermann, however, denies that this is the case, and considers that the injection does not aggravate an attack, if made during the incubation-period. Of the figures given by Haffkine as to the results obtained with his inoculations, we may quote those relating to the village of Undhera.[1] Among 64 uninoculated persons, there were 27 cases of plague, and 26 of these proved fatal; while among 71 inoculated persons—members of the same families as the former and living under exactly the same conditions—there were 8 cases, 3 of which were fatal. The deaths among the uninoculated thus exceeded those among the inoculated by 89·65 per cent.

Leuman[2] records that of 1,173 mill-hands, 1,040 were inoculated twice: among these there were 22 deaths (2·11 per cent.); of 58 inoculated once, 8 died (13·79 per cent.); of 75 not inoculated, 20 died (26·6 per cent.). Bannermann states that in a total of 6,000 cases the mortality among the inoculated was 43·5 per cent., while among the uninoculated it was 73·7 per cent.

[1] *Lancet*, 1899, i. 1697.

[2] Quoted by Miss Slaughter, *Johns Hopkins Hosp. Bull.*, Nov., 1903, p. 307.

Among 7,182 inoculated municipal labourers[1] 14 cases of plague occurred (0·19 %), with 13 deaths (0·18 %); whereas among 418 uninoculated there were 28 cases (6·7 %) and 26 deaths (6·2 %).

Haffkine[2] sums up the results so far obtained in the following figures:—*Inoculated*, 186,797; 3,399 or 1·8 % attacked with plague; 814 or 0·4 % died. *Uninoculated*, 639,630—attacks 49,433 or 7·7 %, deaths 29,733 or 4·7 %.

The Indian Plague Commission reported as follows with regard to this method of prophylaxis:—

(1) Inoculation sensibly diminishes the incidence of attacks of plague. It is, however, not an absolute protection against the disease.

(2) The death-rate is markedly diminished by its means, not only the incidence of the disease but also the fatality (case-mortality) being reduced.

(3) The protection is not conferred, on those inoculated, for the first few days after the injection.

(4) The duration of the immunity is uncertain, but it seems to last for a number of weeks, if nor for months.

The mode of action of Haffkine's prophylactic is presumably the same as that of other vaccines, viz. it depends for its efficacy on the presence of the actual bacteria contained in it. It has, therefore, generally been supposed that the precipitate that forms in tubes of the vaccine which are allowed to stand, consisting of the bodies of the dead bacteria, is the effective part of the preparation. Klein[3] has recently thrown some doubt on the inert nature of the supernatant fluid. He finds that it has a certain, though small, protective influence on rats. Further, he finds that the blood of immunised animals is agglutinative towards the *B. pestis*, but not bactericidal.

[1] *Ann. Rep. Sanit. Commissioner*, 1905.

[2] *Bull. Inst. Past.*, vol. iv. 825.

[3] *Thirty-first Annual Report of the Local Government Board*, 1901-2; Supplement containing the report of the Medical Officer, 1903, pp. 357-394.

The following is the judgment set forth in the *Annual Report of the Sanitary Commissioner with the Government of India* for 1904 : "That its value is great is certain ; not only does it largely diminish the danger of plague being contracted, but, if it fails to prevent the attack, the probability of a fatal event is reduced by one-half" (p. 107). In the Report for the following year (1905) it is stated that the use of the prophylactic has no ill effect on health and that a marked protective influence lasts for six or even twelve months (p. 122).

Wurtz and Bourges, from experiments on white mice,[1] find that the protective power of the prophylactic is considerable, and lasts for a moderate period of time (two or three months). Haffkine considers that the protection afforded by his prophylatic lasts as long as six months. The general opinion in India is that it is "absolutely safe for three months." Leuman found that the protection gained by those twice inoculated was 10 per cent. greater than that of the once-inoculated.

Pfeiffer[2] considers that the bacilli lose some of their virulence by being cultivated in broth, and that their efficacy as a protective is thus diminished. He has accordingly prepared a vaccine from fresh cultures of *B. pestis* on agar. These are emulsified in broth or salt-solution, and sterilised at 65° C. The reaction produced by injection of Pfeiffer's preparations is more intense than that seen after Haffkine's prophylactic. No statistics are available for forming a judgment as to the value of this vaccine as compared with Haffkine's.

Kolle and Strong[3] suggest vaccination with living attenuated bacilli, and Strong has actually carried out this method at Manilla without any untoward occurrences.

[1] *Arch. de Méd. Expérimentale*, &c., 1902, p. 145.

[2] *Deut. med. Woch.*, March 15, 1906, 413.

[3] Quoted by Marx, "Diagnostik Serumtherapie und Prophylaxe," p. 81.

TERNI AND BANDI'S VACCINE

Terni and Bandi[1] prepare a special material, for use as a vaccine against plague, by injecting guinea-pigs intraperitoneally with plague-bacilli and collecting the inflammatory fluid which is secreted into the peritoneal cavities of the animals. This fluid is sterilised by heating for a short period of time, on each of several consecutive days, to 50°C., and is preserved by the addition of a small proportion of carbolic acid. The inventors claim that by means of this vaccine immunity may be produced in eight to ten hours, and that the blood of persons so treated possesses bactericidal powers.

Havelburg[2] records that this vaccine was used with good effects in Brazil. Pinto[3] also records good results with anti-plague vaccinations (with this remedy!): out of 1,803 persons vaccinated only two contracted plague, and one of these cases occurred immediately after the vaccination. He considers the results of the treatment to be brilliant, but it must be remembered that the plague in Brazil was apparently of a mild type. Kolle and Otto[4] regard Terni and Bandi's vaccine as quite inert.

LUSTIG AND GALEOTTI'S VACCINE

The material used for the preparation of Lustig's serum (p. 176) may be employed for the purpose of vaccinating against the disease. It is prepared by growing the bacilli in broth and then on agar. The bacteria are then washed off and dissolved in a 1-per-cent. solution of caustic potash, and the fluid is neutralised with 1-per-cent. acetic acid. A precipitate is thus formed, which is highly toxic, containing

[1] *Deutsch. med. Woch.*, 1901.

[2] *Berlin. klin. Woch.*, 1901.

[3] Abstr. in *Journ. of the American Med. Assoc.*, 1902, i. 681. The nature of the vaccine used is not stated in the abstract. We have been unable to obtain the original article (*Tidsskrift f. d. Norske Laegeforen.*, Feb. 1, 1902).

[4] *Deutsch. med. Woch.*, July 9, 1903.

as it does the intracellular poisons of the bacilli. It is dried *in vacuo*, and can be readily preserved in this form. For use as a vaccine, it is dissolved in a weak solution (1 or 2 per cent.) of sodium carbonate. The dose for an adult is 0·0133 grm. of solid substance. Two grammes of the solid dissolved in 1 litre of solution will afford material for 143 vaccinations.[1] Statistics as to the use of this vaccine are not available.

OTHER VACCINES

Klein[2] has produced immunity in animals by injection of an emulsion of the dried organs of an animal dead of plague, an average guinea-pig yielding 5–7 grms. of dried powder, equivalent to 400–600 doses for an adult rat. A very similar procedure has been devised by Mallannah.[3] Besredka[4] advises the use of bacilli agglutinated with anti-plague serum and suspended in salt-solution.

YERSIN'S SERUM

Preparation of the serum.—The original method of Yersin, Calmette, and Borel[5] for the preparation of anti-plague serum was by inoculation of horses with fresh agar-cultures of the bacilli. It was subsequently found by Roux and Wladimiroff that as effective a serum could be obtained by injection of cultures sterilised by heat, by which proceeding the danger attending the use of living organisms could be avoided. The serum is difficult to prepare of adequate strength, and attempts at its manufacture are at times unsuccessful. Krumbein, Tavel and Glucksmann[6] took a year and a half in attaining a sufficiently active serum. Six months is the time usually found necessary for the preparation of the serum at the Paris

[1] Deutsch and Feistmantel, *Impstoffe u. Sera*, Leipzig, 1903.
[2] See *Brit. Med. Journ.*, 1906, p. 155.
[3] *Centralbl. f. Bakt.*, 1906, Bd. xli. *Orig.*, Heft. 5 and 6; *Lancet*, 1907, i. 222.
[4] *Rousski Vratch*, Oct. 19, 1902.
[5] *Ann. de l'Inst. Pasteur*, 1895, p. 590.
[6] *Centralbl. f. Bakt.* 1901, p. 742.

institution. Before the serum is finally drawn off for use, the blood of the horse is tested on mice to ascertain that no living bacilli are contained in it. One-tenth of a cubic centimetre of serum should protect a mouse from a dose of living bacilli which kills a control-mouse in two or three days.

Value of Yersin's serum.—Yersin gives the following account of his experiences in Amoy.[1] Twenty-three cases were treated in all. Of these—

Six cases treated on the first day, all recovered within 24 hours. Dose, 20–30 cc. No suppuration occurred.

Six cases treated on the second day, all recovered within 3–4 days. No suppuration. Dose, 30–50 cc.

Four cases treated on the third day, all recovered within 4–5 days. Dose, 40–60 cc. Two suppurated.

Three cases treated on the fourth day, all recovered within 5–6 days. Dose, 20–50 cc. One suppurated.

Four cases treated on the fifth day, two died. Dose 60–90 cc.

In Nhatrang (Annam)[2] out of 33 cases treated with the serum, 19 recovered and 14 died (mortality, 42 per cent.); of 39 cases not treated, all died (100 per cent.).

Calmette and Salimbeni[3] used the serum in Oporto. They report that, of 142 cases injected with the serum, 24 died, a mortality of 14·78 per cent.; among 72 patients not so treated, 46 died, a death-rate of 63·72 per cent. They find that the serum reduces the pain in the bubo and limits the inflammation; suppuration is often aborted by its early use.

Cairns,[4] as the result of experience of the remedy in cases at Glasgow, concludes that—

1. Yersin's serum is a remedy of the greatest value.

2. Its action is bactericidal—as shown by the degeneration induced in the bacilli—as well as antitoxic.

3. Good results are best secured by the early administra-

[1] *Ann. de l'Inst. Pasteur*, 1897, p. 81.
[2] *Ibid.*, 1899, p. 251.
[3] *Ibid.*, 1899, p. 865.
[4] *Lancet*, 1903, i. 1287.

tion of large doses, subcutaneously, into the area from which lymph drains towards the bubo, and also intravenously.

4. In mild cases the subcutaneous method alone is sufficient, but in severe attacks combined subcutaneous and intravenous administration is advisable. The total combined dose in the latter condition should be 150 to 300 cc., the proportion given intravenously varying with the severity of the attack.

Valassopoulo [1] used the serum supplied by the Pasteur Institute (Paris) in 100 cases; among 64 treated within the first three days of the disease the mortality was 10 per cent.; among 36 treated on the fourth day and later it was 47 per cent. The mortality among 42 cases not treated with serum was 35 per cent., but in this class were included many very mild attacks. Valassopoulo agrees with Roux that the serum is antitoxic, not bactericidal.

Dose and administration of the serum. — From what has just been said it may be seen that large doses of the serum are to be employed, if the amount is available. Yersin appears to give doses of 20 to 90 cc. according to the date at which the case comes under treatment. He administers the remedy subcutaneously. Cairns uses still larger amounts (150 to 300 cc.), and gives the serum both subcutaneously and intravenously; and the advantages of employing large doses are also enforced by Duprat.[2] Brownlee [3] insists on the intravenous use of the serum, and advises doses of 60 cc.; Lignières [4] gives the same advice. There is no reason to fear the use of the larger amounts. The only ill effects recorded have been pains in the joints and erythema, noted by Calmette and Salimbeni, analogous to those associated with diphtherial and other antitoxines.

Choksy [5] advises an initial injection of 100 cc., followed

[1] *Bull. Soc. Méd. des Hóp.*, 1908, p. 541.
[2] *Ann. de l'Inst. Pasteur*, Sept. 25, 1903.
[3] *Lancet*, Aug. 17, 1901.
[4] *Ann. de l'Inst. Pasteur*, 1901, p. 808.
[5] *Report on the Treatment of Plague*, Bombay, 1906.

by two others of the same amount at intervals of 6 to 8 hours. These may be supplemented by subsequent injections of smaller quantities.

Denys and Tartakovsky [1] insist on the importance of local injections of the serum into the neighbourhood of the buboes. Thus, in cases of inguinal buboes, the remedy should be injected into the leg. They found that if guinea-pigs were inoculated intraperitoneally with plague bacilli, 0·1 cc. of serum injected into the peritoneal cavity would act as a protective dose; whereas 10 cc. administered subcutaneously was of no avail in saving the lives of the animals.

Prophylactic use of the serum.—Yersin records that in Nhatrang no cases of plague occurred among those who had received prophylactic injections of the serum. Calmette and Salimbeni also used the serum as a protective, giving doses of 5 cc. injected under the skin of the abdomen. According to these observers the protection only lasts fifteen days, so that it is advisable to repeat the injections at the end of this time. Calmette recommends the injection of some of the serum along with the use of Haffkine's prophylactic, in order to counteract the first depressing effects of the latter. This suggestion seems worthy of serious consideration when the prophylactic is used in the actual presence of an epidemic. Serum-rashes, joint-pains, and hyperhidrosis may follow the use of this serum.

LUSTIG'S SERUM

Antitoxic serum.—Yersin's serum appears to be bactericidal in nature, though it may possess some antitoxic power. Lustig considers that the curative serum for plague should be mainly antitoxic, and he therefore proceeds to obtain such a preparation by immunising horses with the vaccine-material already described (p. 172), which consists of a poisonous bacterio-protein. The immunising process lasts two or three weeks.

[1] *Semaine Méd.*, 1900, p. 40.

Lustig and Galeotti[1] record that among 475 cases of plague treated with the serum the recovery rate was 39·36 per cent., whereas among 5,952 patients not so treated the recoveries were only 20·6 per cent. Choksy[2] puts the rate of recovery after use of the serum at 38·2 per cent., while in other patients not so treated it was only 19·5 per cent. In another series of cases, 480 patients were treated with the serum, and the same number without it. Eliminating various sources of error, he found that the recoveries among the serum cases amounted to 39·62 per cent., whereas among the non-injected cases they were only 20·21 per cent. The following table shows the results obtained in India with this remedy (Choksy):—

TABLE SHOWING RESULTS OF TREATMENT OF PLAGUE WITH LUSTIG'S SERUM. BOMBAY, 1898–1901.[3]

Period.	Serum-treated Patients.			Patients under Ordinary Treatment.			Difference in favour of the Serum-patients percent.
	No.	Deaths	Case-Mortality, per cent.	No.	Deaths	Case-Mortality, per cent.	
May to Oct., 1898...	257	145	56·4	752	595	79·1	22·7
Jan. to April and June, 1899... ...	189	124	65·60	884	734	83·03	17·4
May, 1899, and July, 1899, to Aug., 1900	484	329	68·00	484	385	79·5	11·5
Aug., 1900, to Feb., 1901 (3 extra cases)	55	36	65·45	184	144	78·26	12·81
March, April, and May, 1901	104	81	77·82	102	81	79·42	1·53

[1] *Brit. Med. Journ.*, Jan. 16, 1901. [2] *Lancet*, 1900, ii. 291.

[3] Choksy, "The Treatment of Plague with Professor Lustig's Serum," Bombay, 1903, p. 110.

Mayr[1] gives an account of 361 cases treated with the serum, the recovery-rate being 33·8 per cent.; while among patients treated by other methods only 21·3 per cent. survived. He says that the general recovery-rate in hospitals where the serum was used was 4·5 per cent. higher than in those where it was not employed. He considers that the curative properties of the serum are definitely established.

A mere study of the above records does not produce a very favourable impression of the value of the remedy. The results obtained do not seem so striking as those seen with Yersin's preparation. More experience is, however, needed to enable us to form a judgment. The opinions of those who have used Lustig's serum, as quoted above, appear to be favourable.

AGGLUTINATION OF BACILLUS PESTIS

Plague-bacilli, like those of enteric fever and many others, are agglutinated by the serum of patients who have just suffered from the disease, or of animals which have been inoculated with the bacilli or their products. There is some difficulty in performing the test owing to the normal occurrence of the bacilli on nutrient media in closely-adherent masses; if, however, the emulsion is made in sterile water and well shaken with glass beads, the bacilli become thoroughly separated. Klein[2] advises that they should be grown on gelatine, on which a drier and less sticky culture is formed, and that they should then be suspended in salt-solution. If it be found that the bacilli are still present in clumps and not distributed singly, it is better to make a thick emulsion of them, and to filter it through a double thickness of filter-paper. The microscopic

[1] *Lancet*, 1900, ii. 461.

[2] *Thirty-first Annual Report of L.G.B.*, 1901 2. Supplement containing the Report of the Medical Officer. Appendix B, No. 1, p. 361.

method of examination must be applied, as the naked-eye or "sedimentation" test is unreliable. Emulsions of the bacilli in broth are also to be avoided for this test, as they tend to spontaneous agglutination without the aid of immune serum. Klein recommends a dilution of 1 : 20 for use, and a time-limit of half-an-hour. He finds that the blood of immunised animals, though strongly agglutinative, is not bactericidal. The growth of *B. pestis* is said to be retarded by addition of serum from a plague-convalescent ; this appears to contain a bacteriolytic copula.[1]

In human patients the agglutinative power of the blood does not develop until late in the disease, often not till convalescence is established. The test is therefore useless clinically. It may, however, be valuable as a proof that a specimen of bacillus under examination is *B. pestis*.

CONCLUSIONS

1. *Haffkine's prophylactic* is a valuable means of protection against plague. There is some doubt as to whether its use in the presence of an epidemic is advisable, owing to the possibility of an increased susceptibility being at first produced. The employment of some of Yersin's serum along with the vaccine seems to offer a means of counteracting this depressing effect, if it really exist.

2. Sufficient evidence is not yet available to enable us to decide as to the efficacy of *Lustig's* or of *Terni and Bandi's vaccine*.

3. *Yersin's serum* is of value as a remedy for the disease. It should be given early in the case and in large quantities. Some of the serum should be injected intravenously, the rest subcutaneously into the area of skin which is drained by the lymphatics leading to the bubo. The dose may be from 60 to 150 or even 300 cc.

4. Yersin's serum may also be used *prophylactically* (dose 5 to 10 cc.), but the protection gained is transitory,

[1] Low, *Trans. Grant Coll. Med. Soc.*, Bombay, 1903–4, p. 3.

so that repeated injections are necessary in presence of an epidemic of plague.

5. The claims of *Lustig's serum* as a remedy are less well established than those of Yersin's serum, but some evidence has been adduced in its favour.

6. The *agglutination-reaction* occurs with *B. pestis*, but it is of no use for purposes of clinical diagnosis, as it occurs too late in the disease.

CHAPTER X

ENTERIC FEVER

Causal organism.—The *Bacillus typhosus* was discovered by Eberth in 1881, and is consequently often called Eberth's bacillus. It is found in considerable numbers in the alimentary canal of infected persons, but also exists in large numbers during the first week or so of the disease in the blood of the infected individual; later it is found in the spleen, and in the lymphatic glands of the abdomen. The organisms are excreted in considerable quantities in the urine, as well as in the fæces, and appear in the sputum of cases complicated by lesions of the lungs or larynx. It appears, therefore, that the disease cannot be considered a local infection only, but is of the nature of a septicæmia or general infection (Wright).

Complications.—As in other infective diseases, the complications met with in the course of enteric fever, or during convalescence from it, are largely due to secondary invasion by other organisms which effect a lodgment in tissues worn out by conflict with a primary illness. The hectic temperature met with in the fourth week in severe cases of enteric fever is probably due to the action of pyogenic bacteria; while to these, or, in some instances, to the *Bacillus coli*, are to be ascribed most of the suppurative lesions (periostitis, perichondritis, otitis, epididymitis, &c.) which are seen in the later weeks. Venous thrombosis, so often met with in convalescence, is also to be attributed to pyogenic bacteria. In some cases, however, typhoid bacilli are found in local suppurative lesions, though it cannot be regarded as certain whether they are the primary cause,

or only find a favourable soil in lesions caused by other bacteria. Possibly the *B. typhosus*, when its virulence is somewhat reduced, becomes a pyogenic organism, as Donzello[1] maintains. The cystitis which sometimes occurs, though it is rarely met with apart from catheterisation, may be due to the bacilli contained in the urine.

Toxines of B. typhosus.—Cultures of typhoid bacilli do not as a rule contain any considerable quantity of free toxic matter, but the bodies of dead bacteria are themselves poisonous. Hence the toxines of the *B. typhosus* are generally spoken of as "intracellular." They are very unstable bodies.[2] Chantemesse claims to have succeeded in growing the bacilli in a special medium containing spleen-pulp and bone-marrow, and from this to have obtained a toxine of considerable potency, which he has used for the preparation of an antitoxine.

Aronson[3] also devised a medium in which toxines were formed, but was unsuccessful in preparing an antitoxic serum. Meyer and Bergell[4] were rather more successful with the toxines of bacilli grown in a peptone-meat-broth prepared from the spleens of oxen, and regard the prospect of a curative use of their serum as hopeful.

ANTITOXIC SERUM

Chantemesse's serum.—By means of the toxines prepared as mentioned above, Chantemesse[5] has produced a serum for the cure of enteric fever. It is prepared by inoculation of horses with the toxine in the usual manner. The process of inoculation is a long and tedious one, as very small doses must be employed at first; otherwise the horses may be killed by the toxine. Chantemesse speaks of losing several in the course of his investigations. The

[1] *Lo Sperimentale*, 1901, lv. 670.
[2] Macfadyen, *Brit. Med. Journ.*, 1906, i. 905.
[3] *Berlin. klin. Woch.*, 1907, p.572.
[4] *Ibid.*, May 6.
[5] *La Presse Méd.*, 1901, No. 93, p. 285.

immunisation of the animals was begun in 1896, whereas the experiments with the serum on patients suffering from enteric fever were apparently carried out in 1900 (?). It may be concluded that at least two years were consumed in the preparation of the remedy. The results of this method of treatment, as recorded by its author, are very encouraging. It is difficult to ascertain the average mortality of the disease, as it varies much in severity in different years, the reasons for this variability not being known. In Paris, in the years 1899 and 1901, the death-rate was 18·5 per cent.; in 1901, from January to October, it was 29 per cent. among 371 patients treated in nine hospitals. Chantemesse treated 100 patients by his method, with six deaths. All those who were treated before the tenth day recovered. Of those treated later, three cases died of perforation of the intestine; one (injected on the twenty-first day of the illness) of pneumonia; one (injected on the twenty-fifth day) of hyperpyrexia, and one of a sacral bedsore acquired before admission to hospital. Two subsequent cases, which were injected on admission to hospital in a moribund condition, are not included in the statistics.

According to some recent statistics,[1] while the mortality in fourteen hospitals where the serum was not used amounted to 17·3 per cent. (3,595 cases, with 763 deaths), in the Bastion Hospital, where the serum was used, the mortality was only 3·7 per cent. (712 cases, 27 deaths).

Chantemesse gives charts of some of the cases treated by the serum, showing that the injections are followed by a rapid fall of temperature and improvement in the pulse. The earlier the serum is administered, the more marked is the effect. If the remedy be given before the eighth day in cases of ordinary severity, the disease may be cut short within a period of a few days. Sometimes the first improvement is not maintained, and the temperature rises again on a later day; in such instances a second injection should be given, and may be followed by rapid recovery. The

[1] Chantemesse, *Paris Med. Journ.*, 1906, i. 3.

accompanying charts, modified from those given in Chantemesse's article, show the results obtained in some of his cases (Charts 1 and 2).

Besides the effects on the pulse and temperature, the serum has a beneficial influence on the excretion of urine, which increases in quantity as the pulse and temperature

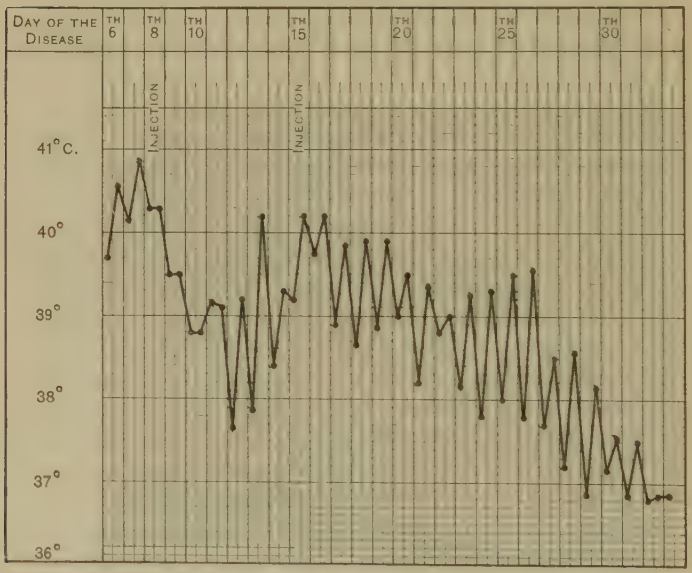

Chart 1. — Illustrating the Effects of Chantemesse's Serum.

fall. Albuminuria is not caused by the serum itself—a point in which it appears to differ from diphtherial antitoxine, which is accused of causing the appearance of albumin—indeed, in cases in which there is already albuminuria, this may decrease as the result of serum-treatment. A hyperleucocytosis is produced in the blood, in opposition to the leucopenia (defective number of leucocytes) which is characteristic of enterica. The leucocytosis is exactly similar to that which is normally

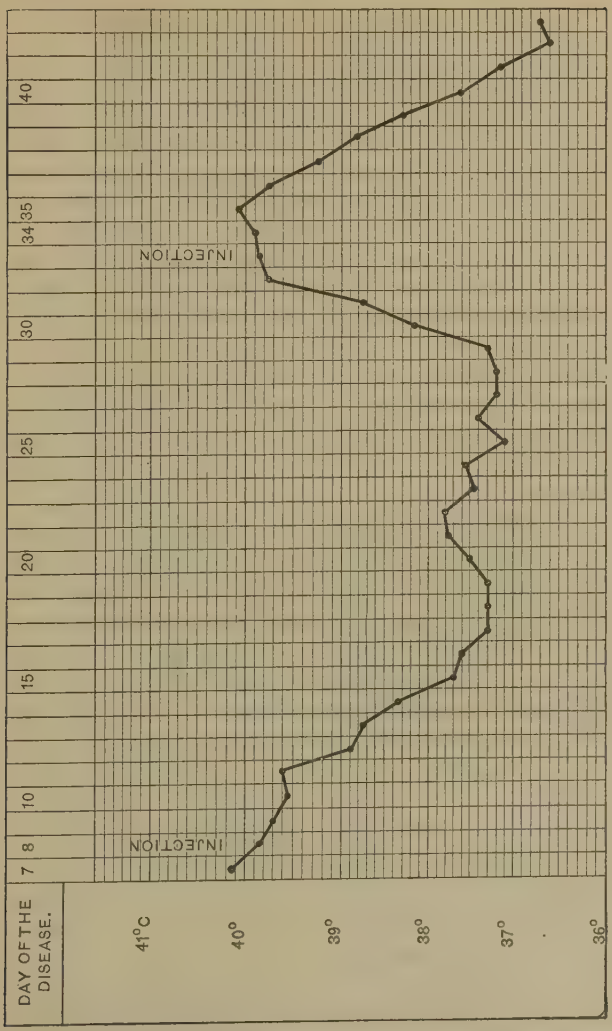

Chart 2.—Illustrating the Effects of Chantemesse's Serum.

seen in convalescents from enteric fever. The myelocytes which are present during the disease disappear, while the other varieties (lymphocytes, eosinophile cells, and

multinucleate leucocytes) increase to their normal amount: they may even be in excess at first.

Complications are rare in cases treated with serum, but are not entirely absent. Chantemesse noted in his first series one case of perforation and one of pneumonia, both fatal; and others of otitis media, hæmorrhage and phlebitis, all of which recovered. Probably the serum has no direct influence on the occurrence of complications: these are due to other organisms, which are unaffected by it; but if it control and cut short the typhoid infection, it must indirectly diminish the risk of secondary lesions.

The serum is injected under the skin of the forearm in the neighbourhood of the usual point for venesection. The skin and the syringe are carefully sterilised, and the veins of the part must not be wounded. The ordinary dose of serum is 10 or 12 cc. A second dose may be given at the end of eight or ten days, if the temperature has risen again, or if there is any other indication; the second dose may be smaller than the first—4 or 5 cc. There are two indications for giving a smaller dose than the above-mentioned as a first injection, viz. (1) when the patient comes under treatment in quite the early days of the disease (fifth or sixth); and (2) when the disease has already lasted for a considerable time, and the general intoxication is profound. In these cases 5 to 8 cc. are sufficient.

The injections are followed by a reaction, shown by a rise of temperature, which quickly falls again. Chantemesse attributes this reaction to the great destruction of the bacilli which is induced by the serum, and the consequent absorption of a large dose of their toxines. He considers his serum to be bactericidal as well as antitoxic. It is difficult to accept this explanation in view of the nature of the serum, which, from its manner of preparation, should be purely antitoxic, and not bactericidal. An additional argument against this assumption may be derived from the

fact that the injection of this serum does not increase the agglutinative power of the blood.[1]

Along with the serum, other treatment should not be omitted, especially reduction of temperature by baths, and the supply of plenty of liquid nourishment to the patient. It may be necessary to stop milk-feeding for a time after the injection, as milk often appears to be ill digested; it may be resumed again as the temperature falls.

No bad effects are produced by the serum, with the exception of slight erythema, which only appeared in two out of Chantemesse's 100 cases.

Besides Chantemesse himself, Boutleux[2] treated 15 cases with the serum; all of these recovered. Josias[3] reports 50 cases in children in which he used the remedy. Among them there were two deaths, a mortality of 4 per cent. Simultaneously, in other children's hospitals in Paris the mortality was 14·2 per cent. He confirms the benefit derived from early administration of the serum, and states that, though relapses occurred in four cases, they were mild in type. The doses given amounted to 1 cc. for each 30 kilogrammes of body-weight. In a certain proportion of cases in young children pain in the bowels was complained of, some days after the injections; and this was at times so severe as to create a suspicion of peritonitis. No ill effects, however, actually ensued. Osteitis and periostitis occurring during convalescence from enteric fever are said to be benefited by the serum.

Before we can pronounce a definite verdict on the value of this serum it will be necessary to wait until a larger number of physicians have used it in the treatment of enteric fever and recorded their results. At present adequate confirmatory experiences are wanting. Chante-

[1] Josias and Tollemer, Congress of Madrid, 1903. *La Presse Méd.*, June 24, 1903, p. 468. [2] Quoted by Chantemesse, *loc. cit.*

[3] International Medical Congress of Madrid. See *Med. Press and Circular*, July 29, 1903, p. 109; *Ann. de Méd. et Chir. Infantiles*, 1903, No. 11, p. 367.

messe's results, however, are very encouraging, although 712 cases form far too small a material on which to base an opinion, since the disease is so variable in its severity at different times and different places.

Other serums.—W. V. Shaw[1] obtained toxic material by "digesting" *B. typhosus* in normal blood-serum. He injected this into a horse and obtained a serum which had some protective power, while the injections were at first followed by a fall in the bactericidal property of the horse's serum—"negative phase." From this it would appear that the serum is antibacterial rather than antitoxic, and hence comparable with that mentioned in the following section rather than with Chantemesse's preparation.

Macfadyen immunised a horse by injecting with endotoxin, prepared by grinding up typhoid bacilli at the temperature of liquid air, and showed that the serum was both antitoxic and bactericidal. Hewlett[2] continued the observations and employed the serum in 9 cases of enteric fever, and concluded that 8 were benefited and 2 of these decidedly shortened. Goodall[3] tried it in 26, and Bruce[4] in 5 cases; benefit being observed in 9 of these last 31 cases.

BACTERICIDAL SERUM

Antityphoid serum.—The serum of convalescents from enteric fever is bactericidal and not antitoxic in its action; and most of the serums on the market, which are professedly "antityphoid," are of similar nature. They are prepared by immunising a horse with the actual bacilli of enteric fever. Chantemesse[5] alludes to experiments made by Widal and himself in order to produce a serum of this nature, but he speaks of the results obtained as unsatisfactory. In 1898, Bokenham[6] prepared an antityphoid serum

[1] *Lancet*, Oct. 3, 1903, p. 948.
[2] *Proc. Royal Soc. Med.*, 1909, ii., Medical Section, p. 245.
[3] *Op. cit.*, p. 254.
[4] *Op. cit.*, p. 262.
[5] *Loc. cit.*
[6] *Trans. Path. Soc. Lond.*, 1898, p. 373.

by inoculating a horse with filtered cultures of the bacilli and then with the dead bodies of the organisms themselves; he found that the serum acted as a protective to rabbits.

Walker[1] describes the method adopted by Krumbein, who uses first filtered cultures, then bacteria killed by carbolic acid. The bacilli are grown for fourteen days in broth, to which $\frac{1}{2}$ per cent. of phenol is then added. The cultures are injected subcutaneously, and considerable constitutional disturbance may be produced. Abscesses may also form at the seat of injection. After a point had been reached at which 150 cc. were given for a dose, the serum of the horse was drawn off and used. In subsequent experiments the living bacilli were injected during the later periods of the immunising process.

It appears that the typhoid bacillus is modified to some extent by its surroundings, and that different strains of bacteria thus produced, taken from different cases, may act variably towards a particular serum. In other words, a serum is found to have a more marked effect on the strain of bacilli from which it was prepared. It is, therefore, advisable to make use of several strains in the immunisation of the horse. The serum of the horse becomes highly agglutinative towards the bacilli as the result of the treatment. Walker considers that the agglutinative power increases practically *pari passu* with the protective property, but that the two are not directly proportional to one another. He finds that the serum prepared as above is antitoxic as well as antibacterial. He also makes the suggestion that the horse should be immunised against the *B. coli communis* as well as against the *B. typhosus*, or that some "anticoli" serum should be added to the antityphoid serum for therapeutic use.

Experience at the present day is not very favourable to the use of an antibacterial serum in the treatment of enteric fever. The present writers have seen the ordinary serum

[1] *Journ. of Pathol. and Bacteriol.*, 1901, p. 251.

which is on the market tried in several cases of the disease, but in none of them was it possible to be sure of any definite benefit accruing to the patient. Relapse was not prevented by the use of the serum. Walker concludes that "most antityphoid sera which have been prepared have given no marked assistance in the treatment of the disease in man." Reasons for this have been already suggested. Moreover, in the later stages of the disease the bacilli are for the most part localised in the alimentary canal, and the toxines are probably absorbed, as in cholera, without any further escape of the organisms into the general circulation. If so, it may be difficult for the antibacterial serum to reach them; and attention should be turned to the preparation rather of an antitoxic serum than of one that is germicidal. In the second place, it may be that the copula or immune body present in horse-serum is not capable of uniting with the alexine or complement found in human blood, in which case no bacteriolysis would be produced. It has further to be remembered that enteric fever is characterised by a gradual onset, so that it is seldom recognised until it has lasted at least five or six days. Hence the first requisite in the administration of any kind of serum—early injection—is generally impossible, and it is unreasonable to expect as good results to occur as can be obtained in diphtheria. It is possible that by the time the serum is used there may be a deficiency of complement in the patient's blood, and that bacteriolysis may not occur, even if the copula supplied be suitable. Finally, if the destruction of the bacilli is brought about by the interaction of the serum with the leucocytes of the patient, the leucopenia characteristic of the disease may militate against the efficacy of the remedy.

ANTITYPHOID EXTRACT OF JEZ

Jez[1] starts with the assumption that the serum obtained from immunised animals is bactericidal and not antitoxic, and that such a serum is of no value for the treatment of

[1] *Wien. med. Woch.*, Feb. 18, 1899, p. 346.

enteric fever. Some other method of conferring immunity must be tried. Now, Wassermann found that the spleen, bone-marrow, and lymphatic glands of an immunised animal had protective properties; and Jez has made use of this discovery to prepare a substance which he considers to be curative of enteric fever. He makes his antityphoid extract by rubbing up in a mortar the brain, spinal cord, spleen, marrow, &c., of immunised rabbits, and adding to the pulp thus obtained saline solution, to make an emulsion, along with a small amount of alcohol and of carbolic acid. The fluid is filtered after it has stood for a time, to ensure solution of the protective bodies. In later experiments Jez added also a certain proportion of pepsine, presumably in order to facilitate solution.

The filtered fluid is antitoxic, but not agglutinative or bacteriolytic. As a remedy for enteric fever, it is given by the mouth; but if for any reason this is impossible, it can be administered subcutaneously. A tablespoonful constitutes a dose, which may be given every two hours, or more frequently. Considerable quantities are needed for each case, reaching a pint or more.

Jez finds that, as the result of treatment with his extract, the temperature falls, the pulse improves, and the general condition of the patient is ameliorated. Diarrhœa is usually checked. Sometimes sweating is produced by the action of the remedy. Jez records the trial of the extract in eighteen cases, all of which recovered.

These results are confirmed by Kluk-Kluczycki,[1] who finds that the fever-reducing effect is manifested within twenty-four hours, an apyrexial condition being often reached within three weeks. Eichhorst[2] has also tried the extract in a small number of cases (twelve), and is favourably impressed with the results produced; and a similar verdict is pronounced by du Mesnil de Rochemont[3]

[1] *Wien. klin. Woch.*, 1901, No. 4, p. 84.
[2] *Therap. Monatsh.*, 1900, p. 115.
[3] *Ibid.*, Jan., 1904, p. 13 (7 cases).

and by Einhorn,[1] who observed a reduction of fever and some mental improvement. On the other hand, Pometta[2] found Jez's preparation quite useless.

The use of Jez's extract does not seem to have become at all general, so that there is not sufficient information available upon the subject to enable us to form a satisfactory judgment as to its efficacy. The idea underlying it is not to be neglected, as Wassermann's experiments, confirmed by Jez, seem to point to the existence of a protective principle in the organs of immunised animals (*cf.* p. 173, Plague). This does not, however, necessarily imply its value as a cure for the disease.

ANTITYPHOID INOCULATION

Wright's vaccine.—Experiments were made by Pfeiffer and Kolle[3] in 1896 as to the effect of inoculating patients with cultures of typhoid bacilli; but although it was shown that the blood of those so treated had a protective influence on guinea-pigs, no practical use seems to have been made of the method. It is to Wright that the practical introduction of vaccination as a means of prophylaxis against enteric fever is entirely due.

The vaccine originally used by Wright[4] consisted of cultures of *B. typhosus* in broth, grown for four weeks, and then sterilised by heating for ten to fifteen minutes at 60° C. For this vaccine a large number of separate cultures are mixed together so as to obtain a fluid of the standard strength. Special flasks also are used for the preparation of the cultures, in order to facilitate the subsequent mixing. The virulence of the material can be roughly gauged by its opacity to light, for the measurement of which Wright devised an ingenious arrangement. A small amount of carbolic acid or lysol is subsequently

[1] *Med. Record*, Jan. 16, 1904, p. 81 (3 cases).
[2] *Wien. med. Woch.*, 1901, No. 46.
[3] *Deut. med. Woch.*, 1896.
[4] *Lancet*, 1901, i. 150.

added to ensure sterility and the preservation of the vaccine.

The dose used for an injection in man is the minimal lethal dose for a guinea-pig weighing 100 grammes, or rather the proportional fraction of the dose which proves fatal to one of the ordinary weight (250 to 300 grm.). A virulent culture will contain the requisite quantity in 0·5 cc., but with weaker vaccine it is necessary sometimes to give as much as 1·5 cc. Wright also used a vaccine consisting of agar-cultures of the bacilli grown for twenty-four hours and sterilised at 60° C.: these are less toxic than the broth-cultures.

The injections are followed by redness and pain at the site of inoculation, with some lymphangitis and enlargement of neighbouring glands. There may be nausea and even vomiting, and there is considerable feeling of illness, with some rise of temperature. Occasionally a condition approaching collapse is observed. These symptoms pass off rapidly without leaving any permanent ill effects, but they are severe enough to act as a very real deterrent.

Wright subsequently found that better results were obtained if the vaccine was prepared from 24-hour cultures grown upon the surface of agar, and, after emulsification, standardised to contain 1,000 millions of typhoid-bacilli in every cubic centimetre. Sterilisation is effected by heating for half-an-hour at 56°–58° C., and ½ per cent. lysol is afterwards added. The dose is 500 or 1,000 millions of bacilli. Two injections, the first of 500 and the second (fourteen days later) of 1,000 millions, are however preferable. We frequently give *three* doses, the first two of 500 millions, separated by an interval of ten days, and the third of 1,000 millions, eight days after the second. After such injections the local reaction and neighbouring lymphangitis are the only resulting inconveniences.

The immediate result of the vaccination is to produce a lowering of the resistance offered by the individual to infection by enteric bacilli. If large doses of the vaccine are

given, this fall in immunity may be very marked, and may last for some weeks. If small doses are given, the fall in resistance is very slight and transitory. For these reasons it is advisable to make use of small doses, repeated if necessary, rather than one large dose. It is also important not to vaccinate in the presence of an epidemic, as such a procedure would tend to make the subject more liable to contract the infection.

Almost all the statistics as to the efficacy of Wright's vaccination are derived from observations on different units of the British Army in South Africa during the Boer war, and in India, Egypt and Malta. On the following page are some of the figures given by Wright himself.

Cayley [1] also gives favourable figures with regard to the use of inoculation in the members of the Scottish National Red Cross Hospital. Among 57 inoculated persons in the 1st Section no attacks occurred; among 82 of the 2nd Section, the greater number were inoculated with old vaccine, and five orderlies developed enteric fever; one nurse refused inoculation, and she also suffered. Among the 3rd Section (20) all were inoculated, and no cases of the disease occurred. Cayley considers that cases which do occur in inoculated persons are milder and run a shorter course than in the uninoculated.

Birt [2] quotes his experience in an epidemic at Harrismith. Among 947 unvaccinated patients the mortality was 14·25 per cent, while of 263 who had been inoculated, the death-rate was only 6·8 per cent. These figures point to the disease being of a milder character in those who have been vaccinated. Kuhn [3] used three inoculations, and found that no "negative phase" followed the third. He records good results, and states that immunity lasts for

[1] *Brit. Med. Journ.*, Feb. 9, 1901.

[2] *Ibid.*, Jan. 11, 1902. *Cf.* Ward, *Journ. R.A.M.C.*, 1906, vi. 431.

[3] Abstr. *Centralbl. f. Bakt.*, 1907, xl. 602. *Cf.* Eichholz, *Münch. med. Woch.*, 1907, 777.

Statistics of Antityphoid Inoculation.[1]

Group.	Total Numbers.	Cases of Enteric Fever.	Percentage Incidence.	Deaths.	Percentage Death-rate.	Case-Mortality.
British Army in South Africa	Inoculated ... 4,502	44	0·98	9	0·2	1 in 4·9
	Uninoculated ... 25,851	657	2·54	146	0·56	1 in 4·5
15th Hussars	Inoculated ... 360	2	0·55	1	0·27	1 in 2
	Uninoculated ... 179	11	6·14	6	3·25	1 in 2·2
Garrison of Ladysmith ...	Inoculated ... 1,705	35	2·05	8	0·47	1 in 4·7
	Uninoculated ... 10,529	1,489	14·14	329	3·12	1 in 4·5
Garrison of Egypt and Malta	Inoculated ... 720	1	0·14	1	0·14	1 in 1
	Uninoculated ... 2,669	68	2·55	10	0 37	1 in 6·8
British Army in India, 1900	Inoculated ... 5,999	52	0·87	8	0·13	1 in 6·5
	Uninoculated ... 54,554	731	1·69	224	0·58	1 in 3·3
British Army in India, 1901	Inoculated ... 4,883	32	0·66	3	0·06	—
	Uninoculated ... 55,955	744	1·33	199	0·36	—
City Imperial Volunteers	Inoculated ... 700	60	8·5	9	1·3	1 in 6·7
	Uninoculated ... 494	39	7·9	11	2·2	1 in 3·5

[1] A. E. Wright, *The Practitioner*, March, 1904, p. 370. The groups consisting of the largest numbers are here selected. For complete statistics reference may be made to the original article.

one year. Bassenge and Mayer [1] suggest for purposes of vaccination the use of a clear fluid prepared according to Brieger's method by shaking typhoid-bacilli with distilled water and filtering. The preparation is very stable, but immunity only lasts about three months.

It must be remembered that one attack of enteric fever does not protect against a second, as was at one time supposed. Experience in South Africa has definitely proved that second attacks are not by any means rare. Hence vaccination cannot be expected to produce absolute immunity. But second attacks seem, as a rule, to be comparatively mild in degree, and it is probable that a similar degree of protection may be looked for in the inoculated. On the whole, so far as the evidence at present available enables us to draw a conclusion, it would seem that persons going into parts of the world where enteric fever is very prevalent would do well to be inoculated. There is some reason to think that the protection afforded is less marked in those who are past the age of thirty [2]; but it is also probable that individuals become progressively less susceptible as they advance in life; hence the above advice applies chiefly or entirely to those below that age. The temporary inconvenience [3] caused by the injections cannot be held to constitute a sufficient disadvantage to counterbalance the protection gained. We repeat that those who are actually in the midst of an epidemic of enteric fever should not be inoculated, owing to the increased liability to contract the disease which at first ensues as a result of the treatment.

[1] *Deut. med. Woch.*, May 4, 1905.

[2] Crombie, *Lancet*, Aug. 16, 1902.

[3] Occasionally the symptoms produced by the original vaccine were severe and even alarming, though no fatal results are recorded. Thus, Lindsay (*Lancet*, 1905, ii. 827) saw a case in which an acute illness ensued, lasting fourteen days; and we have seen a similar case, in which there was very high fever (103°–104° F.) for several days, with headache, sleeplessness, and distension of the abdomen, following the second of two injections given at an interval of seven days.

VACCINE-TREATMENT

Petrushky [1] has made experiments with a preparation which he calls *typhoïn*, consisting of dead bacilli. He reports good results in cases of uncomplicated enteric fever, if the remedy is given early in the course of the illness. It is not suitable for patients in whom the disease is advanced and in whom there is already a tendency to heart-failure or general intoxication. Small, gradually increasing doses are given, and the first injections are accompanied by some antityphoid serum, to prevent ill effects.

Pescarolo and Quadrone [2] have used a vaccine of living typhoid-bacilli upon patients suffering from the disease, and noted good results—fall of temperature and rapid recovery. They made use of agar-cultures attenuated by heating to 45°–50° C. The inoculation is followed by shivering and rise of temperature, while some redness and swelling may appear at the site of injection.

Semple [3] also reports favourably on the effects of vaccine-treatment in this disease. He used doses of 15–30 millions of bacilli daily for four to six days, and controlled the results by opsonic estimations. He recommends the employment of cultures derived from the individual patient.

AGGLUTINATION-REACTION

"Widal's test."—This reaction was first suggested as a practical test for the diagnosis of enteric fever by Widal in 1896, although experiments in this direction had previously been made by Grünbaum; the latter were not published till after Widal's communication. The "test" may therefore fairly be called "Widal's," although he was not the discoverer of the phenomenon (*see* p. 13). A very large amount of experience is now available as to the occurrence of the "reaction" in cases of enteric fever. It was at first

[1] *Deut. med. Woch.*, 1902, p. 212.
[2] *Centralbl. f. inn. Med.*, 1908, No. 40, p. 989.
[3] *Lancet*, 1909, i, 1668.

thought that the mere occurrence of agglutination, produced by addition of serum to a vigorous culture of typhoid-bacilli, was sufficient to prove that the person from whom the serum was derived was, or had been, suffering from enteric fever. It was, however, soon discovered that the serum of normal individuals may produce this effect, if it be added in sufficient strength. A dilution of the serum to one part in ten was next adopted as the standard, but this again was found unsafe. Then a 1 : 20 standard was substituted. For practical purposes this dilution is of considerable value, but it is now recognised that no absolute diagnosis can be made as to the existence of enteric fever on a positive reaction occurring with a less dilution than 1 : 30, or even, according to some authorities, 1 : 50. With the stronger mixtures a time-limit of half-an-hour is customary; with the weaker some observers prolong the time of observation to two hours. Libman [1] states that a positive reaction may sometimes occur in high dilutions (1 : 50) when it is not present in more concentrated mixtures (1 : 20); he therefore recommends the use of two dilutions for each test.

Mode of performing the test.—The blood of the patient may be obtained from either the finger-tip or the lobe of the ear. The latter is, perhaps, the better of the two, as it is rather less sensitive, and the blood flows quite as freely, if not more so. The skin should be cleaned up first with lysol or some similar antiseptic, and then with sterilised water: this precaution is not, however, absolutely necessary. The lobule of the ear is then firmly grasped with the fingers of the left hand, and a deep puncture is quickly made with a sharp surgical needle, or with a special instrument made for the purpose. A common needle will serve, if no other is available. The blood is collected, as it exudes, in a glass bulb drawn out at either end into a fine point; the ends being sealed in a flame after the blood is collected. In the tube coagulation takes place, and the serum which exudes from the clot is ready for use.

[1] *Med. News*, Jan. 30, 1904, p. 204.

Several different ways of effecting the necessary dilution of the serum are employed. It is best to use, at all events for the higher dilutions, a graduated pipette, which saves time and trouble. Sterile broth is used for the purpose, or some neutral fluid such as normal saline solution. The broth-culture of the *Bacillus typhosus* must be a recent and vigorous one, in which the bacilli are moving freely about. In older cultures an agglutinating substance is formed by the bacilli and diffuses out into the liquid; in such specimens the bacilli are found to have become clumped without the addition of any extraneous material, and are therefore unfit for use. The addition of a few drops of an old culture to a young and vigorously moving emulsion will produce agglutination. It is well to observe the condition of the culture before using it, in order to see what (if any) degree of clumping is already present.

When the dilution has been made and the bacilli added to it, a drop of the mixed fluid is placed on a cover-glass, and a hanging-drop preparation is made and observed under the microscope. The cover-glass should be ringed round with vaseline or some similar substance, to prevent evaporation. A high power is not necessary; indeed, it may even be a source of fallacy to beginners, by leading them to mistake the small clumps which are present in almost all cultures for the larger masses which form as the result of the true agglutination. If a true agglutination of the bacilli takes place, it will be seen that almost all of them have run together into masses, while any that remain free have lost their motility and remain stationary in the field of the microscope.

The test may also be done macroscopically, by mixing the serum and culture in a test-tube or watch-glass. A visible precipitate falls if the reaction is positive. According to Berliner and Cohn [1] a star-like figure is seen in a watch-glass in half-an-hour at room-temperature.

[1] *Münch. med. Woch.*, Sept. 11, 1900.

McWeeney[1] has devised a special method of performing the test, by growing the bacilli in hanging drops, one with the serum to be tested, the other with normal serum. If the reaction is positive, the bacilli in this drop will be seen to form chains and to be non-motile, whereas in the "control" experiment they are separate and freely motile. The serum is added in the proportion of 1 per cent., and the slides are kept at 37° C.

Hewitt and Rowland[2] are the authors of a means of performing an exactly graduated quantitative test. The serum is received into capillary tubes, of which the thickness of the walls and the diameter of the lumen are measured under the microscope, while the length of tube which is filled by the serum is easily ascertained. In this way the exact volume of serum is calculated, and subsequent dilution is effected by measured proportional amounts of broth.

Ficker[3] has devised a method of performing the test with dead bacilli, specially prepared and suspended in an indifferent fluid, the nature of which has not been published. For use the serum to be examined is diluted (1 : 10) with saline solution, and mixed with the slightly turbid test-fluid. If the reaction is positive, the mixture becomes clear, a slight precipitate falling to the bottom. Ten to fourteen hours are allowed for the reaction to take place.

The value of this ("*Ficker's diagnostic*")[4] as a test is confirmed by Meyer,[5] Ehrsam,[6] Sadler,[7] and others. It is however, not so delicate as Widal's test performed in the ordinary way.[8] If further experience prove favourable, the discovery should afford a useful means of applying the

[1] *Dublin Journ. of Med. Science*, Sept., 1898.

[2] *Brit. Med. Journ.*, April 28, 1900.

[3] *Berlin. klin. Woch.*, 1903, p. 1021.

[4] Ficker's diagnostic may be obtained from Merck, of Darmstadt.

[5] *Berlin. klin. Woch.*, 1904, p. 166.

[6] *Münch. med. Woch.*, 1904, p. 662.

[7] *Berlin. klin. Woch.*, 1905, No. 10.

[8] Güttler, *ibid.*, 1904, Nos. 51, 52; Selter, *Münch. med. Woch.*, 1905, No. 3.

test, as the dangers inseparable from living organisms and the trouble of preparing fresh cultures will be avoided. The preparation is said to keep well for at least nine months.

Sources of error.—(1) Apart from the possibility of error just alluded to, owing to a spurious appearance of agglutination, it must be borne in mind that a certain number of normal individuals, who have never suffered from enteric fever, possess a serum with some clumping power over typhoid-bacilli, while not all patients suffering from the fever present the reaction. Thus Lobiesen[1] found that, out of 350 cases which were clinically enteric fever, 328 reacted positively to the Widal test with a dilution of 1: 50; 17 agglutinated in dilution of 1 : 10 or 1 : 25; two cases reacted only at 1 : 5; and two failed to react at all. The great majority of the patients (289) gave a positive reaction within the first two days after admission. Of 151 patients suffering from diseases other than enteric fever, in whom there was yet a suspicion that the malady might be of this nature, four reacted positively in dilution of 1 : 25, two at 1 : 10, and 123 were negative at this dilution. Of the first four, three were proved by necropsy to be suffering respectively from acute tuberculosis, retroperitoneal abscess, and calculous pyelitis with tubercular meningitis. Of 61 healthy persons who had not had enteric fever, one reacted positively in dilution of 1 : 25, and eight at 1 : 10. Lobiesen believes that a positive reaction in a dilution of 1 : 50 is pathognomonic of enteric fever; but that absence of the agglutination does not exclude the existence of the disease. Blanchi,[2] among 167 patients with enteric fever, found three cases which did not react to the test. The diagnosis was confirmed by necropsy.

Rolleston[3] in South Africa found that the test was positive in only 64·5 per cent. of his cases, and thinks that the low percentage may be accounted for by some difference in the

[1] *Zeit. f. klin. Med.*, Bd. xliii., Hft. 1 and 2.
[2] *Giornale Medico del Regio Exercito*, 1901, No. 5.
[3] *Brit. Med. Journ.*, Oct. 12, 1901.

strain of bacilli used for the test-cultures, as compared with the organisms which had infected the patients. Kohler[1] found that among 100 patients suffering from diseases other than enterica 12 gave a reaction in dilutions of 1 : 20. Among these 12, one agglutinated at 1 : 50, two at 1 : 40, and three at 1 : 30. Hence it appears that even an agglutination-reaction in a dilution of 1 : 50 is not a definite proof of the existence of enteric fever.

(2) Again, it has been frequently noticed, and the present writers have had many opportunities of observing, that in some severe and fatal cases of enteric fever, in which the diagnosis is subsequently confirmed by *post-mortem* examination, no agglutinative power is found throughout the illness. The absence of agglutination is parallel to the absence of resistance exhibited by the patients towards the infective agent.

(3) The clumping power of the serum is not developed at the beginning of the attack. The exact period at which it may be looked for is not certain, but many observations have shown that during the first week or even ten days an absence of agglutinative power is rather the rule than the exception. Brion and Kayzer,[2] however, find the reaction positive in the first week in 50 per cent. of all cases.

(4) Certain other infective and general diseases are apparently capable of producing substances in the serum which will agglutinate typhoid-bacilli. Infection with *B. coli communis* seems to produce this effect in some cases.[3] Allusion has already been made to the experiments of Posselt and Sagasser,[4] who found increased clumping power towards *B. typhosus* in cases of dysentery.

Morgan[5] found that of six cases of cerebro-spinal

[1] *Münch. med. Woch.*, Aug, 13, 1903, p. 1379.

[2] *Deut. Arch. f. klin. Med.*, lxxxv., Hft. 5 and 6.

[3] *Cf.* Lubowski and Steinberg, *Deut. Arch. f. klin. Med.*, 1904, p. 396 (proteus-infection, staphylococci, &c.).

[4] *See* p. 15.

[5] "An Account of an Outbreak of Spotted Fever, &c." Swansea, 1909.

meningitis, three gave a "Widal" reaction when the serum was diluted 1 : 50, and one produced a definite but less marked agglutination.

The effect of jaundice in causing agglutination was first pointed out by Grünbaum,[1] and has been confirmed by other observers. The reaction does not seem to be manifested in all cases of jaundice, but it may occur with sufficient frequency to cause us to regard the test as unreliable when this condition is present. Bile itself does not appear to agglutinate the bacilli in all cases, but in certain individuals and conditions of health it may produce the reaction. The exact body which is active in this way is not known, but its effect is analogous to the action of formalin and other chemical substances. It must, however, be borne in mind that infection of the biliary passages with *B. typhosus* is not uncommon, and that the jaundice may be a result of this—the occurrence of the reaction thus being the "exception that proves the rule."

(5) All strains of *B. typhosus* are not agglutinated with equal facility. It is therefore necessary to make use of a culture in which the bacilli have been proved to possess this faculty. Klein finds that culture of the bacilli on gelatine produces greater agglutinative properties, and advises the use of such cultures emulsified with salt-solution.

(6) Kraus[2] finds that the effect of pneumonia is to inhibit the agglutinative power. This may be demonstrated by adding the serum of a pneumonic patient to that of one suffering from enteric fever. The coexistence of pneumonia with enteric fever would, therefore, theoretically prevent the appearance of Widal's reaction, and the test would be useless as a means of differentiating the two diseases. These observations, however, need further confirmation. Kissel and Mann,[3] on the other hand, found that two cases of

[1] *Münch. med. Woch.*, 1897, No. 13.
[2] *Zeit. f. Heilk.*, Bd. xxi., Hft. 5.
[3] *Münch. med. Woch.*, May 2, 1899.

croupous pneumonia gave the reaction, though they were not suffering from enteric fever.

Value of the test.—On the whole it may be said that the reaction is not infallible, but has a margin of error of perhaps 5 per cent. Abbott[1] studied 4,154 cases, and found the error only 2·8 per cent. We believe that, with a time-limit of thirty minutes, a positive reaction in a dilution of 1 : 20 implies either the existence of enteric fever or a past attack within recent years: a reaction in a dilution of 1 : 50 implies either existing disease or a very recent attack; and a reaction in a dilution of 1 : 200 is positive proof of present typhoid infection.

The test is said to be of special value in the case of children. Gershel,[2] among 84 cases of enteric fever in infants, found the reaction positive in 81, while it was negative in 115 patients who were suffering from other diseases. Other writers also agree with this estimate of the use of the test in children.[3]

It is generally stated that the agglutination-reaction is of no use as a means of *prognosis*. This is probably true as far as the rapidity and completeness of the clumping are concerned; but there is reason to think that a case which, clinically, is almost certainly a severe attack of enteric fever, but which gives no reaction, is likely to end fatally, as the absence of agglutinative power in such instances seems to be associated with an absence of resistance.

Persistence of the reaction. — H. French and Louisson[4] find that as a rule the reaction disappears rapidly after the patient's recovery, but that in exceptional cases (7·5 per cent.) it may remain for years (eight years and probably longer). Gachtgens[5] found the reaction positive in one case thirty-five years after an attack.

[1] *Philadelphia Med. Journ.*, Feb. 25, 1899.

[2] *Med. Record*, Nov. 26, 1901.

[3] Josias and Tollemer, *Med. Press and Circular*, Aug. 26, 1903, p. 217.

[4] *Guy's Hosp. Rep.*, 1907, lxi. 235.

[5] See *Lancet*, 1907, i. 1363.

OPHTHALMIC REACTION

Chantemesse[1] suggests as a test the use of a powder derived from the bacilli, which is applied to the conjunctiva and causes inflammatory reaction in patients suffering from enteric fever, just as tuberculin does in tuberculous subjects.

This test has been tried by Kraus, who finds that 60 per cent. of patients with enteric fever react positively. Cohn and Entz find that healthy persons may also give a reaction.[2]

Meroni holds that a negative result after twenty-four hours excludes enteric fever, while a positive reaction raises a probability, but not a certainty, of typhoid infection.[3]

ANTISTREPTOCOCCIC SERUM

Many of the complications of enteric fever are due to secondary invasion by pyogenic and other organisms. In this belief Clarke[4] has tried the effect of antistreptococcic serum in purulent periostitis occurring in enterica. He states that the use of this remedy was followed by a critical fall of temperature and a rapid disappearance of the suppuration.

CONCLUSIONS

1. The bactericidal serum on the market, called "antityphoid," is generally unsatisfactory in its effects in cases of enteric fever—indeed, it is doubtful whether it can be said to have any influence at all on the course of the disease.

2. Good results are reported with Chantemesse's antitoxic serum, but there is not yet sufficient material on which to form a judgment as to its value.

[1] *Deut. med. Woch.*, 1907, p. 1572.
[2] *See* Wolff Eisner, *op. cit.*, *sub* Tuberculin.
[3] *Münch. med. Woch.*, 1908, No. 26.
[4] *Lancet*, Jan. 28, 1899, p. 230.

3. Treatment by vaccines has not yet emerged from the experimental stage, and no opinion can yet be expressed as to its value.

4. The same criticism applies to Jez's antityphoid extract.

5. Wright's antityphoid inoculation confers some measure of protection. It is not a certain means of preventing an attack of enteric fever, but it reduces the liability to this occurrence; while, if the disease does occur in a "vaccinated" person, it is generally milder than in one not so protected. It seems advisable for young persons going to countries where enteric fever is rife to undergo prophylactic inoculation. There is no danger in the procedure, but the immediate effects may be unpleasant.

6. The Widal test (agglutination) for enteric fever is a useful means of clinical diagnosis. It is not infallible, but the margin of error is small—perhaps 5 per cent. An agglutination in a dilution of not less than 1:50 should be obtained before the result is called positive.

PARATYPHOID INFECTIONS

Cases also occur, identical clinically with those of enteric fever, but differing in their etiology, being due to one or other of the so-called "paratyphoid" bacilli (*B. enteritidis* of Gärtner; *B. paratyphosus* of Schottmüller, types α and β; or *B. paratyphosus* of Bryon and Keyser). In such cases the organism can usually be isolated by establishing cultivations in suitable media from blood (5–10 cc.) collected in a sterile syringe from a vein in the arm. The most convenient guide to diagnosis is, however, the agglutination-reaction, for *B. typhosus* is rarely clumped by the serum of these patients in any higher dilution than 1:20 (the clumping then being due to the presence of group-agglutinins), whilst the organism responsible for the infection is clumped in dilutions of 1:100 and 1:200, and even higher.

In cases which clinically resemble enteric fever, but fail to give a positive reaction to Widal's test, the agglutinating effect of the serum should be tried on both the varieties of paratyphoid bacilli (α and β) if these are available.

Franchetti [1] has prepared an antitoxic serum in rabbits by injecting broth-cultures of *B. paratyphosus* β. The serum agglutinates this organism, but has not been used as a remedy in human disease.

[1] *Zeitschr. f. Hyg.*, 1908, lx. 128.

CHAPTER XI

CHOLERA

Causal organism.—The organism which is responsible for the production of cholera is a curved, rod-shaped bacterium, which was discovered by Koch, and is called the *Vibrio choleræ*, or *Spirillum choleræ*.

Toxines.—It has not been possible to prepare any potent solutions of the toxines of the organisms in artificial media: the toxines are therefore classed as "intracellular." Ransom,[1] however, obtained from cultures a solid substance which induced the formation of an antitoxine when injected into goats. Metchnikoff and his assistants also succeeded in obtaining a feebly toxic fluid by growing the vibrios in peptone-water. These preparations can, however, only faintly reproduce the toxines which are formed in cases of the disease of which the virulence is such that in acute cases death may occur within a few hours. The bodies of the vibrios contain within them a substance which produces necrosis of tissue when they are injected subcutaneously.

Macfadyen[2] obtained an antitoxic serum by injecting rabbits and goats with ground-up cultures of the vibrios; it was also agglutinative and bacteriolytic. The endotoxine of the bacteria is thermolabile, being destroyed at a temperature of 55° to 60° C.

Allusion has already been made to Pfeiffer's experiments (p. 8) on the destruction of cholera-vibrios in the

[1] *Deut. med. Woch.*, 1895.

[2] *Lancet*, 1906, ii. 494. *Cf.* Brau and Denier, *Ann. de l'Inst. Pasteur*, 1906, xx. 578; Hahn, *Münch. med. Woch.*, 1906, No. 23.

peritoneal cavity of immune animals. It is found, however, that if a large dose of the bacteria is injected into an "immune" guinea-pig, the animal dies in spite of the destruction of the organisms; in other words, although the bacteria are broken up and killed by the serum, this has no power of neutralising their intracellular toxines, which are set free and kill the guinea-pig.

Agglutination and diagnosis.—The vibrios of cholera are agglutinated by means of the serum of convalescents from the disease, and by that of animals artificially immunised. Karwacki[1] states that agglutination of the vibrios with the serum of a suspected case in a dilution of 1:30 is a satisfactory proof of cholera. The reaction is of no use for the diagnosis of cholera, as it does not occur till late in the disease; it may, however, be employed for the identification of a particular vibrio as that of cholera Asiatica, since there are several organisms which belong to the same group and closely resemble one another. A more certain test is afforded by Pfeiffer's experiment: the organisms in question are injected into the peritoneal cavity of a guinea-pig along with a sufficient quantity of a serum known to be bactericidal towards the *V. cholerœ*, and their fate is investigated. A serum of the above nature may be readily produced by injecting a rabbit subcutaneously with laboratory cultures of the cholera-organisms, and may be preserved by means of 0·5 per cent. carbolic-acid solution.

"Immune" serum is capable of dissolving *V. cholerœ* in a test-tube, when it is fresh, but it rapidly loses the power; this, however, may be regained by the addition of a little fresh normal serum of the same species of animal. In other words, the complement or alexine tends to disappear in course of time, whereas the copula or immune body remains stable.

These experiments on animals are of considerable importance from the point of view of human therapeutics.

[1] *Zeitschr. f. Hyg.*, 1906, 39.

They show that there can be little hope of treating the disease, when already developed, by means of a bactericidal serum; for if the bodies of the bacteria are so toxic in themselves, such a serum, by leading to rapid breaking-up of those vibrios which were already present, could but produce speedier intoxication. The hope of preparing an antitoxic serum is at present slight, as attempts to produce a potent toxine in artificial media have failed, and it is only by means of a toxine of high potency that an effective antitoxic serum can be obtained. Hence it is in the direction of prophylaxis—of inducing a condition of active immunity which shall destroy the bacteria that first gain access to the body before they have increased in numbers up to a dangerous degree—that the best hope of combating the disease seems to lie.

VACCINATION AGAINST CHOLERA

Experimental vaccination against cholera was first carried out by Ferran, who employed broth-cultures of the *Vibrio choleræ* derived from the stools of patients. No definite statistics are available as to the amount of success which Ferran obtained by his inoculations.

Haffkine's cholera-vaccine.—The method in use at the present time for anticholera vaccination is that of Haffkine. This method involves the use of two vaccines, a weaker and a stronger, the former being administered first in order to avoid the destructive effect of the virulent organisms on the tissues at the point of injection. The weak vaccine is obtained by growing the bacteria on agar at a temperature of 39° C. in a current of air. The stronger vaccine is prepared by passing the vibrios through a series of guinea-pigs till a virus is obtained which is invariably fatal to these animals within eight hours. The method adopted is as follows: a guinea-pig is inoculated intra-peritoneally with a laboratory-culture of the organisms, which usually causes death within 24 hours. The peritoneal exudation is collected from the dead guinea-pig, and is

incubated for 10 hours at 35° C., the optimum temperature for the organisms. (This is done in order to give the bacteria time to multiply, as they are only found in comparatively small numbers in the first peritoneal fluid.) After incubation the fluid from the first guinea-pig is injected into the peritoneum of a second, and so on through a series of animals, till the "*virus fixe*" is obtained. This is cultivated for 24 hours on agar-tubes, the whole surface of a sloped-agar tube being inoculated. When growth has occurred the whole culture is washed off with sterile broth, and the quantity made up to 8 cc. One cubic centimetre (one-eighth part) of this constitutes a dose of the vaccine. The virulent cultures soon become attenuated by growth on laboratory media, and must be again raised in virulence by passage through animals.

The injection is given hypodermically in the flank, and an interval of five days should separate the two vaccinations. The procedure is followed by redness, swelling, and pain in the side, and by a febrile reaction. The degree of protection afforded is said to be proportional to the severity of the symptoms. The immunity conferred by each injection is attained in five days; hence the selection of this interval of time between the injections. Before employing the vaccines generally, Haffkine made trial of them on himself and others: no ill effects were produced. He has now given 70,000 injections in 42,179 individuals without accident.

Results of vaccination.—Haffkine [1] gives the following figures relating to his experiences at Calcutta and Lucknow:—

Population.		Cases.		Deaths.	
		Total.	Percentage.	Total.	Percentage.
Non-Inoculated	1735 ...	174	10·63	113	6·51
Inoculated ...	500 ...	21	4·20	19	3·80

[1] *Brit. Med. Journ.*, 1895, i. 219.

Powell [1] also reports favourably on the results obtained with this prophylactic, and gives the following statistics :—

Population.	Cases.		Deaths.		
	Total.	Percentage.	Total.	Percentage.	Fatality.
Non-inoculated 6,549	198	3·02	124	1·89	63%
Inoculated ... 5,778	27	0·48	14	0·24	50%

Out of 275 uninoculated coolies on steamers plying between Goalundo and Dilrugarh, 8·36 per cent. contracted cholera, and 10 died; while of 414 who had been inoculated, only 1·2 per cent. contracted the disease, and none died of it.[2]

In 1903 the figures were as follows :—2,633 non-inoculated, 68 cases, 16 deaths; 199 inoculated, 4 cases, 1 death. In 1904, 614 non-inoculated, 6 cases, 3 deaths; 75 inoculated, no case. The inoculations have, nevertheless, been discontinued by the wisdom of the Government.[3]

It appears from these figures that the use of Haffkine's prophylactic inoculations confers a certain measure of immunity to cholera. Larger statistical material is necessary to enable us to gauge with accuracy the exact amount of protection which it affords. There is reason to believe that this, like other methods of vaccination with pathogenic bacteria, may produce an initial fall in the resistance of the individual to the disease; and therefore it may be questioned whether it would be wise to undergo inoculation in the presence of an epidemic. Complete immunity—so far as it is ever complete—is gained at the end of ten days, in which time two vaccinations have been carried out. Those who are about to visit an infected area, but who can allow this

[1] *Journ. of Tropical Medicine*, 1899, No. 2.

[2] *Ann. Rep. of Sanitary Commissioner with the Government of India*, 1901, p. 88.

[3] *Ibid.*, 1903 and 1904.

period of time to elapse before they are actually brought face to face with the epidemic, would be wise to undergo vaccination. It is possible that a certain measure of protection may be gained by the end of the fifth day, as a result of the first inoculation; but this is probably slight. It is noteworthy that the prophylactic diminishes the liability of the inoculated person to cholera, rather than the fatality of the disease when it occurs in those who have been vaccinated.

Marx points out that an element of doubt exists in the statistics of cholera-vaccination, in that it is the better-informed upper classes who submit to inoculation, while the poorer portion of the population, owing to ignorance and superstition, refuse to avail themselves of the protection offered. It is among the latter that the incidence of the disease may be expected to be greatest and the mortality highest, so that the apparent protection afforded to the vaccinated may really be due to their better circumstances in other respects.

Kolle[1] considers that the necrosing power of the virulent bacilli may be neglected in practice, and that no ill effects may be expected from an injection of virulent organisms without preparatory inoculation with attenuated cultures. He therefore advises only one vaccination with the virulent organisms, thus producing more rapid immunisation. Powell records that Haffkine now uses more virulent cultures for his first vaccination, and that no suppuration or other accident has been noted as a result of this procedure.

Strong[2] has recently devised a mode of vaccination against cholera by means of the body-substance of the spirilla, which he calls "cholera receptors." The vaccine is obtained by "autolytic digestion" of the organisms, *i.e.* by incubating an emulsion of them in sterile water, in which

[1] *Zeitschr. f. Hygiene*, 1894, Bd. xvi. and xviii. *Centralb. f. Bacteriol.*, 1896, Bd. xix.

[2] *American Medicine*, Aug. 15, 1903, p. 272.

they break up spontaneously. The following are the directions given by the writer :—

"The surface of flat-sided flasks filled with cholera agar (*sic*) are sprayed with 20-hour bouillon cultures, and the flasks then put aside in the incubator at 37° C. for 20 hours; the growth is then emulsified with sterile water, removed from the surface of the agar, and the emulsion placed in a sterile flask and kept at a temperature of 60° C. for 24 hours. The mixture is then put aside in the incubator for from 2 to 5 days. The best results were obtained apparently after 5 days autolytic digestion. After such digestion the emulsion is filtered through a Reichel filter. The fluid thus obtained must, of course, be examined for sterility and carefully standardized before being used as a human vaccine."

No trial of the method on man has apparently been made. Strong considers that the disagreeable effects inseparable from the use of Haffkine's vaccine render it unsuitable for general use.

Murata,[1] who inoculated with dead organisms, gives the following figures :—

Inoculated, 10,000, with 6 attacks and 42 per cent. fatality; uninoculated, 10,000, 13 attacks and 75 per cent. fatality.

PASSIVE IMMUNITY TO CHOLERA

The protection conferred by Haffkine's prophylactic is of the nature of active immunity, the blood of the patient gaining bactericidal power, and thus destroying those organisms which first enter the body and tend to cause infection. It has already been mentioned that the prospects of obtaining a bactericidal or antitoxic serum as a cure for the disease are not hopeful. Popoff[2] vaccinated a cow with comma-bacilli, and found that the milk contained a protective substance which conferred some degree of immunity on guinea-pigs. This substance was destroyed by boiling the milk.

[1] *Centralbl. f. Bakteriol.*, 1904, xxxv., No. 5.
[2] *Vratch*, 1893, No. 10.

CONCLUSION

Haffkine's prophylactic inoculation confers a certain measure of protection against cholera, and should be employed by those who are called upon to reside in districts in which they will be exposed to infection. It should probably not be performed in the actual presence of an epidemic, owing to the increased susceptibility induced during the first few days after the injection.

CHAPTER XII

AFFECTIONS DUE TO STREPTOCOCCI

STREPTOCOCCIC SEPTICÆMIA, RHEUMATISM, CHOREA, SCARLATINA, PNEUMONIA, GONORRHŒA, CEREBRO SPINAL MENINGITIS

Nature of the organisms. — Much controversy has centred round the question of the identity or diversity of the streptococci found in different conditions. Recent writers[1] have enumerated eighteen different (?) species. An attempt was at one time made to differentiate them into "short" and "long" varieties, viz. those which formed chains consisting of a large number of separate cocci, and those which occurred in smaller groups; but this classification is untenable, as the same coccus may form short or long chains according to the circumstances of its environment. Within the body of an infected animal these organisms generally occur in pairs, or as separate units, only showing chain-formation in artificial media. Thus, the pneumococcus (*Diplococcus pneumoniæ*) may take the form of chains of cocci when grown outside the body. There appears to be no essential difference with regard to chain-formation between streptococci and many so-called diplococci.

Many observers hold that all the streptococci found in different pathological conditions are in reality the same organism, modified in some of its characteristics by circumstances. A strong upholder of this view is Marmorek,[2] who has examined organisms derived from erysipelas, puerperal fever, scarlatina, pustules and boils,

[1] Andrewes and Horder, *Lancet*, 1906, i. 1245.

[2] *Ann. de l'Inst. Pasteur*, March, 1902, *et passim.*

and sore throats, and finds that all of them produce the same poison, and that all are antagonised by an antitoxic serum prepared from cultures of any one of them. Aronson[1] is of the same opinion; he finds that a horse immunised against one variety is resistant to all.

On the other hand Meyer,[2] using the method of agglutination, believes that there are two different species: (1) the pyogenic organism, met with in erysipelas, suppuration, &c.; and (2) a streptococcus met with in cases of angina (sore throat). Andrewes and Horder[3] distinguished five different types, and Foulerton[4] also believes in the existence of more than one species, these writers basing their views upon the changes produced in solutions of various carbohydrates.

At present the question of the unity or diversity of the streptococci cannot be answered with certainty. It is clearly of the greatest importance. For the moment we have these two clinical facts strongly before us—viz. that since the introduction of "polyvalent" antistreptococcic serum, there has been a marked improvement in therapeutic value, and further, that frequently when the serum prepared by one manufacturer has proved entirely without effect, the employment of antistreptococcic serum from another maker has been immediately followed by a complete amelioration of the patient's condition. It seems difficult to believe that the pneumococcus, which has, besides other peculiarities, the property of forming a capsule, is not a separate species of organism. Other diseases in which chain-cocci have been met with are rheumatism, chorea and scarlatina. We may consider the questions of serum-treatment connected with these organisms under the following heads: (1) Septicæmia and Erysipelas; (2) Rheumatism; (3) Scarlatina; (4) Pneumococcic Infections; (5) Gonorrhœa; (6) Cerebro-spinal Meningitis.

[1] *Deut. med. Woch.*, 1903, June 18, p. 439.

[2] *Ibid.*, 1902, No. 42. For experiments on agglutination of streptococci, *see also* Zelenski, *Wien. klin. Woch.*, 1904, p. 406.

[3] *Loc. cit.*

[4] *Lancet*, 1904, ii. 1828.

1. SEPTICÆMIA AND ERYSIPELAS

Streptococcus pyogenes.—This organism was first described by Ogston in 1881 and by Rosenbach in 1884. It has been found in cases of spreading cellulitis and pyæmia, in some instances of malignant endocarditis, in puerperal sepsis, in erysipelas, in the pyogenic affections complicating acute infectious diseases (small-pox, enteric fever, &c.), in some membranous sore throats, in a variety of intestinal lesions, and in the cutaneous affections known as ecthyma and impetigo contagiosa. Marmorek[1] has prepared a special medium for growing streptococci, consisting of ordinary broth, peptonised meat, leucine, and glycocol. He has also grown them in a mixture of broth and human serum, and in broth mixed with serous fluid from the pleura or peritoneum. By cultivating them in this way and alternately passing them through rabbits he has succeeded in producing bacteria of such virulence that 0·00000000001 cc. of the culture will inevitably kill a rabbit. This quantity is calculated to contain on an average one single streptococcus.

If any pathogenic streptococcus is grown in a fluid medium until growth ceases, no other strain of these organisms will subsequently flourish in the same fluid without addition of further nutrient material. This test is used by Marmorek to prove the identity of all the streptococci.

Toxines.—Marmorek finds that all streptococci, whatever their origin, manufacture the same toxine. It is of the nature of a diastase, and its activity is destroyed at a temperature of 70° C. In addition to the diastatic ferment, streptococci give rise to a poisonous substance which has hæmolytic powers; this has been called "streptocolysin," and is said to possess a toxophore and a haptophore group.[2] It is destroyed at a temperature of 70° C. Its presence

[1] *Ann. de l'Inst. Pasteur*, vol. ix., No. 71; also 1902, p. 169.

[2] Ruediger, *Journ. of the Amer. Med. Assoc.*, Oct. 17, 1903.

accounts for the petechial eruptions, and perhaps for the jaundice, so often met with in septic conditions.

ANTISTREPTOCOCCIC SERUM

The serum originally introduced by Marmorek was prepared by inoculating horses with the virulent organisms obtained in the way previously recorded. Living cultures are more efficacious in the formation of a potent serum than are dead bodies. Very small doses are used for the first injections, which are gradually raised as the animal becomes more resistant : the final dose may reach 600 cc. of a virulent culture. The process extends over a year. Each injection must be sufficient to produce a distinct reaction ; and the blood of the horse is withdrawn four weeks after the last injection. Marmorek finds that it is impossible to produce an effective serum by means of the toxines of the cocci alone ; apparently these, as obtained in artificial media, are not strong enough. He considers that his serum has a weak antitoxic power ; it is chiefly bactericidal. Raw,[1] on the other hand, maintains that the Paris serum is mainly antitoxic.

Many authorities, however, hold that the passage of streptococci through animals reduces their virulence for man, and that a fundamental error is inherent in Marmorek's method of preparing his serum. Of the different brands of serum now in use those of Marmorek and Denys are made from organisms passed through animals, and those of Tavel, Krumbein, Moser and Menzer from streptococci derived from human sources. Aronson combines both kinds. Experiments by Meyer and by Sommerfeld appear to show that of these serums Aronson's is the most effective.[2]

Value of antistreptococcic serum.—A few general considerations as to the use of antistreptococcic serum may

[1] *Lancet*, 1898, July 9.

[2] *Zeitschr. f. klin. Med.*, 1900 ; *Centralbl. f. Bakt.*, 1903, xxiii.

be set out at this point. In the first place, it must be remembered that cases of infection with pyogenic organisms differ from one another in severity almost more than do attacks of any other kind of disease. It is, therefore, practically impossible to form any estimate of the mortality of such cases from statistics, and equally impracticable to apply the statistical method to recorded cases of cure or failure. In cases which appear hopeless, recovery may take place in a most marvellous manner without the use of any specific remedy. If such a "cure" is effected in a case treated with serum, there is naturally a tendency to ascribe the good result to the serum. On the other hand, there are many instances in which the infection is so intense and so rapid in its onset that it would be hopeless to expect any antibacterial serum to prove efficacious. Only a very large collection of cases would thus serve as a basis for statistical calculation, and this the nature of the disease makes it specially difficult to procure, as most instances of invasion by pyogenic organisms may be described as "accidental"; that is to say, they are sporadic in occurrence and due to inoculation of cocci in wounds, &c., while the state of health of the individual attacked appears to play a greater part in the process than in most other diseases. We are thus deprived of the aid afforded by the epidemic occurrence of disease, in which large numbers of cases are met with, under very similar circumstances, are often treated in special hospitals, and are readily adapted for collection and tabulation. Finally, many of the series of cases reported are of a very heterogeneous nature, embracing, for example, puerperal fever, erysipelas, and cellulitis, the writer applying the results obtained in all of these together to establish the value of the serum in a general way. It will be best briefly to consider the opinions that have been expressed as to serum-treatment in each of these conditions separately.

The exact mode of action of antistreptococcic serum is not satisfactorily determined. It is not bacteriolytic *in*

vitro. Menzer[1] believes that it acts by inducing phagocytosis (opsonically), but Reisch[2] denies this in the case of Aronson's serum.

Puerperal fever.—A special committee was appointed by the American Gynæcological Society to consider the value of antistreptococcic serum in puerperal sepsis. The committee reported[3] that they had collected records of 352 cases treated with serum, among which the mortality was 20·74 per cent., whereas among all cases of the disease not so treated the total death-rate worked out at 5 per cent. It would seem on the face of this report that the committee must have concluded that the use of the serum had increased the mortality of puerperal fever by about 15 per cent.—a fair instance of proving too much, since no one can reasonably maintain that deleterious effects are often due to the serum, and this the committee themselves admitted; the most that can be said against it is that it is ineffectual. Hence the only conclusion that can be fairly drawn from the above figures is that the statistical method is untrustworthy in this instance. The report of the committee is valuable as affording a very complete bibliography of cases recorded up to the time of its appearance.

Savor[4] records his results in sixteen cases: in six no good effects were seen, in five the value of the remedy was doubtful, in the remaining five good results were obtained.

Blumberg[5] tried the serum in twelve cases, all of which were severe, and in nine of which a bacteriological examination was made of the lochia.

Two cases showed anaërobic diplococci; no good effects from serum.

Four cases showed mixed infection, some streptococci; two died, one improved after injection.

[1] *Münch. med. Woch.*, June 23 and 30, 1903. *Cf.* Zangemeister, *Deut. med. Woch.*, July 5, 1906.

[2] Abstr. *Centralbl. f. Bakt.*, 1906, xxxviii. 72.

[3] *American Journ. of Obstetrics*, 1899, xl.

[4] Quoted by Blumberg (*see* below).

[5] *Berlin. klin. Woch.*, 1901, No. 5, p. 132.

Two cases showed sterile lochia; both recovered, a fall of temperature occurring after injection.

Two cases showed pure streptococcal infection; both recovered.

Three cases (lochia not examined); two showed fall of temperature after injection (one already convalescent).

Blumberg is favourably impressed as to the value of the serum. Peham[1] also records good results in cases of pure streptococcic infection; in mixed infections and cases due to other organisms no good results can be expected. Burkard[2] treated 29 cases of pure streptococcic infection without a death. Falkner[3] records 83 cases with 14 deaths, and thinks the serum useful; and Bumm[4] also noted favourable results, using Aronson's serum. Pilcer and Ebersen[5] speak favourably of Marmorek's serum as an adjuvant to other methods of treatment; but McMurtry[6] believes all serum-treatment of puerperal septicæmia to be quite useless. Many isolated cases of benefit attributed to the remedy in puerperal sepsis are recorded, but on the whole the results seem to have been disappointing. We can only conclude that further observation is needed to establish its true position, but that it is advisable to give the serum a trial in cases in which streptococci are found.

Erysipelas.—Marmorek states that he treated 423 cases of erysipelas with his serum, with good results, the mortality being 3·87 per cent. Denys[7] reports good effects from local injections of the serum into the neighbourhood of the affected area. He gives four injections of 0·25 cc. around the lesion. Mayer and Michaelis,[8] and Ayer,[9] noted improvement in cases treated with serum.

[1] *Wien. klin. Woch.*, 1904, p. 405.
[2] *Arch. f. Gynäk.*, Bd. lxxix., Hft. 3.
[3] *Wien. klin. Woch.*, 1907, p. 1582.
[4] *Berlin. klin. Woch.*, Oct. 31, 1904.
[5] *Therapeut. Monats.*, 1904, p. 509.
[6] *Brit. Med. Journ.*, 1906, ii. 1207.
[7] *Semaine Méd.*, 1901, p. 40.
[8] *Berlin. klin. Woch.*, 1903, p. 377.
[9] *Med. Record*, Aug. 26, 1905.

The serum of convalescents has been tried by Fornaca[1] with apparent benefit, but does not seem to be a practicable remedy. Diphtherial antitoxine has also been recommended.

Cellulitis and septicæmia.—Thomas[2] records a series of fifteen cases of sepsis successfully treated with the serum; he administers doses of 30 cc. Good results are reported by Marmorek and others. One of the present writers had a personal experience of the use of serum in his own case, when he was suffering from spreading cellulitis due to a poisoned *post-mortem* wound. The subjective effect was certainly good, the feeling of illness and mental disturbance lessening concomitantly with its use. The temperature also fell gradually, but it is impossible to be sure whether this was attributable to the serum or to the surgical measures adopted. In this case the serum was administered in doses of 10 cc. every four hours for several days. No ill effects were noticeable, but for some time after the injections there was very intense itching at the sites of injection, and faint lines of pigmentation marked the tracks made by the needle.

Ulcerative endocarditis.—Some good results have been obtained in this disease by the use of the serum. Thus, two cases are recorded by Mitchell Bruce[3] in which apparent cure resulted. In one of these a certain brand of serum was found ineffectual, only a temporary fall of the fever being observed; but on using a serum prepared by a different maker, permanent improvement was effected. This case, which came under the notice of the present writers (*see* Chart 3), is instructive as illustrating one reason for the want of success which has often been experienced with this serum. Such failure may be due, apparently, to the existence of various strains of the cocci: each strain gives rise to a serum which is effectual against that particular variety, but which does not equally lead to the destruction of

[1] *Policlin.*, July, 1905.
[2] *Journ. of American Med. Assoc.*, Feb. 18, 1899.
[3] *Lancet*, Aug. 20, 1898, p. 515.

other strains of streptococci derived from different sources (*see also* below, Staphylococci).

Ogle [1] also speaks favourably of the use of the serum in malignant endocarditis, and Dyce Duckworth [2] records a case in which *rectal* administration of the serum apparently effected a cure. Raw,[3] again, saw good results from this method of treatment, 20 cc. of serum being given with

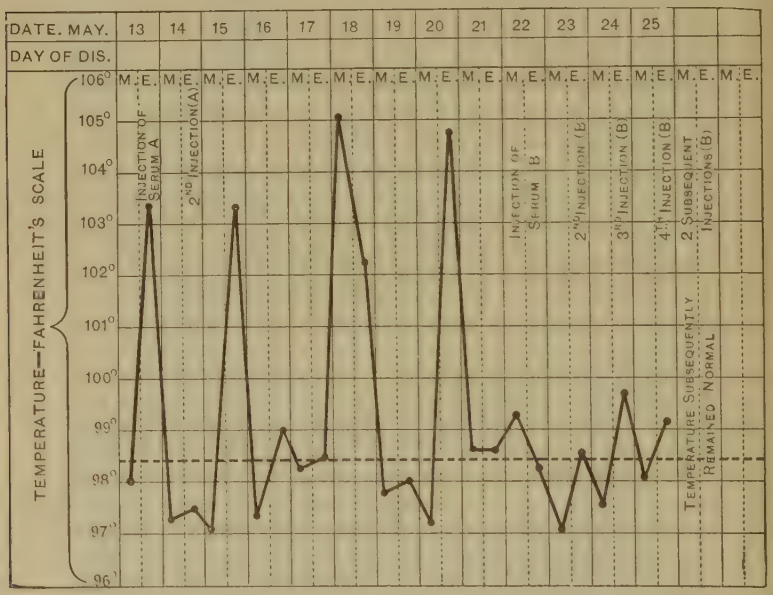

Chart 3.—Illustrating the Efficacy of a second Brand of Antistreptococcic Serum after Failure of the first.

40 cc. of hot saline solution. Ward [4] collected a total of twenty-five cases, of which eight were cured and three temporarily improved.

[1] *Lancet*, March, 14, 1903.
[2] *Brit. Med. Journ.*, May, 23, 1903.
[3] *Lancet*, 1906, i. 1103. *Cf.* Fenwick & Parkinson, *ibid.*, 1244.
[4] *Albany Med. Annals*, Oct., 1903, p. 515.

Pernicious anæmia.—Hunter,[1] relying on his discovery of streptococci in cases of pernicious anæmia, treated some patients suffering from this disease with antistreptococcic serum. He considered that good results were produced. In one case of this disorder, which was admitted to Charing Cross Hospital, the use of the serum was followed by collapse and death, which appeared to be due to the injection, and may have been analogous to the few recorded instances of death occurring after diphtherial antitoxine. It was, however, impossible to be certain that the remedy was actually accountable for the death of the patient, as pernicious anæmia is a disease which causes profound degeneration of the cardiac muscle, and it is difficult to be certain of the exact degree of this myocardial disease in an individual case. Sudden death may occur from asystole at any time. The rapid onset of delirium and coma after the injection was, however, remarkable.

Gangrenous stomatitis.—Cahall[2] reports a case of this affection successfully treated with the serum. Diphtherial antitoxine may be useful in cases of noma due to *B. diphtheriæ*.

Purpura hæmorrhagica. — Coutts[3] successfully treated two cases of this disease by rectal injections of antistreptococcic serum. He expresses, however, some doubt whether normal (horse-) serum would not have been equally effective (*see* p. 351).

The use of antistreptococcic serum in small-pox and in phthisis is alluded to elsewhere (*see* pp. 152 and 303).

Local collections of pus.—In cases in which actual collections of pus have formed, the use of the serum can do no good, so far as the local lesion is concerned. Surgical measures for the evacuation of the pus must be adopted. Subsequently the serum may perhaps prevent the formation of other foci of suppuration, or the death of the patient from

[1] *Lancet*, Feb. 10, 1900, p. 374.
[2] *Philadelphia Med. Journ.*, Feb. 17, 1900.
[3] *W. London Med. Journ.*, 1908, p. 286.

septicæmia. Menzer considers that, if pus be present, the serum may even do mischief, as it may cause a breaking-up of a large number of the streptococci, and so produce an intoxication of the whole system by absorption of poisonous products. This is possibly true, if local surgical measures are neglected.

Ill effects of the serum.—Horse's serum being the basis of antistreptococcic serum, the same by-effects may occur after its employment as after diphtherial antitoxine, viz. erythematous and urticarial eruptions and pains in the joints. Allusion has just been made to one fatal case that was apparently due to the serum.

General considerations.—It is impossible to resist the conclusion that on the whole the use of antistreptococcic serum has been disappointing. This may be due to many causes. In the first place, the existence of several strains of the cocci, which react differently to a given serum, introduces a constant source of failure, especially if only one brand of serum is available for use. In cases which are sufficiently chronic to admit of the trial of a second brand in the event of primary failure, this course should be adopted, and may prove successful.

In the second place, all antibacterial serums appear to keep badly, quickly losing their bacteriolytic power. Hence only freshly-prepared serum should be employed. Many of the serums on the market have at one time or another been found to be inert. Some of the disappointments recorded may have been due to neglect to secure a freshly prepared serum.

Again, many cases of sepsis are not due to streptococci alone, but are complicated by the presence of other germs. Although the antistreptococcic serum may be able to counteract the former organisms, yet the others may have gained so firm a footing as to prove fatal to the patient.

Finally, there can be little doubt that, until lately, recourse was seldom had to serum-treatment till the disease was too far advanced. Owing to the difficulty of procuring the serum and the expense involved, the remedy was only

administered as a last resource. We have seen that in the case of all serums the most important condition for success is early use. Now that a more plentiful supply of serum is obtainable, and that the medical profession is becoming more acquainted with the value of these remedies, it may be hoped that better results will be recorded.

We cannot doubt that in a certain proportion, at all events, of the cases recorded, the serum has acted most beneficially. It is impossible to know beforehand, on any general grounds, which cases will react to the remedy and which will show no improvement. It would seem, therefore, advisable in all cases in which infection by streptococci threatens to become generalised (septicæmic) to have recourse to the serum at as early a stage as possible. No harm is likely to result in any case, and a fatal septicæmia may be warded off.

No assistance can be expected from antistreptococcic serum in cases due to infection by other pyogenic organisms —staphylococci, *B. pyocyaneus*, &c. It is, therefore, advisable to ascertain, if possible, whether any individual case is due to the presence of streptococci, before using the serum. For this purpose from five to ten cubic centimetres of blood should be withdrawn, preferably from a vein in the arm, by means of a sterile syringe, and distributed amongst several tubes or flasks of suitable fluid culture-medium ; or the pus from any local lesion which is available may be examined for the cocci; or the streptococci may be sought for in the urine, by which channel they are often excreted. In cases, however, in which a bacteriological examination is not immediately possible, it is preferable to inject the serum at once, if the symptoms are severe and point to streptococcic infection, rather than wait any considerable time for the bacteriological report.

DIPHTHERIAL ANTITOXINE IN SEPTIC CONDITIONS

Diphtherial antitoxine has now been tried in a large number of differentc onditions besides diphtheria, and

benefit has been ascribed to its use. Paton [1] considers that it is almost specific for septic conditions generally. He concludes (1) that it is specific for all affections due to streptococci and staphylococci, and for simple traumatic inflammations: (2) that it acts as an absorbent for inflammatory tissues and for effused blood; (3) that it has an influence on the coagulability of the blood; and that (4) it acts beneficially in depressed nervous conditions (which may perhaps be due to septic or autotoxic conditions).

Paton considers that the serum acts well when it is given by the mouth, and he adopts this method of administration. He gives the following formula:—

℞. Diphtherial antitoxine, 3,000 units; Trag. carmin., a sufficiency; water to 2 ounces.

One fourth part of this (750 units) is given night and morning, or every four hours. In erysipelas the dose is administered every eight hours. In acute peritonitis or perityphlitis it is given at once, and repeated in two hours' time; then, again, every four to six hours. The serum may cause slight renal irritation or cutaneous eruptions, but these effects are unimportant.

More recently Paton appears to state that the amount of units of antitoxine given is immaterial, and that the normal serum of the horse or ox is almost equally effective (*see* p. 351), the process of immunising the animal against diphtherial toxines only calling out in greater amount some body always present in the animal's serum, which stimulates the cells of the human body to resist microbial attack.[2]

In the present state of our knowledge it appears difficult to take very seriously any claims as to the value of an antitoxine in other diseases than that for which it is prepared. It may possibly produce some leucocytosis, and may thus be beneficial, but it is at least as probable that

[1] *Australasian Med. Gaz.*, Feb. 20, 1902.

[2] "New Serumtherapy," London, 1906.

the cures attributed to it are instances of the fallacy of mistaking *post hoc* for *propter hoc*.

SUMMARY OF TREATMENT WITH ANTISTREPTOCOCCIC SERUM

After adopting such surgical measures as are indicated—

1. Inject 20 cc. of polyvalent antistreptococcus serum, at least half intravenously.

2. If marked improvement occur, repeat the dose in 24 hours.

3. If no obvious improvement be noted, administer a similar dose of polyvalent serum prepared by another manufacturer.

4. If there be still no improvement, try a third brand.

VACCINE-TREATMENT

Recently, attempts have been made to treat affections due to streptococci by means of vaccines consisting of dead organisms. It does not seem probable that much good can result from this procedure in cases of general septicæmia, though Sutcliffe and Baily [1] attribute the cure of one such case to the use of a vaccine, the dose rising from 10,000,000 to 50,000,000 streptococci.

Barr and Douglas [2] record the apparent cure of a case of ulcerative endocarditis by means of a *vaccine* prepared from the actual organism derived from the patient. Horder [3] also tried this method, but without success.

In localised infections with streptococci good may be effected. Thus Weinstein cured post-operative fistulæ by this method.[4]

In a case of appendicitis in which œdema occurred after operation in the neighbourhood of the wound,

[1] *Lancet*, 1907, ii. 367.
[2] *Ibid.*, 1907, i. 499.
[3] *Lancet*, July 16, 1904.
[4] *Berlin. klin. Woch.*, 1906, No. 39.

Hawkins and Corner[1] successfully employed inoculations of a vaccine of *Streptococcus fæcalis*, the organism present. Inoculations of a vaccine of *B. coli* were employed simultaneously, but Wright, who carried out the opsonic and bacteriological investigations in the case, considered that this organism played at most a subsidiary part.

From what has already been said regarding the various species of streptococci it would appear probable that stock vaccines would be useless in the treatment of generalised streptococcic infections, and in practice this is found to be so—the vaccine employed must be prepared from that species actually infecting the patient. Further, as time is of such great importance, every device that will shorten the period spent in preparing the vaccine must be adopted. Consequently cultivations from septic material should be planted directly on to blood-agar plates, and if these are carefully observed during incubation, it will usually be found possible, even when several different kinds of bacteria are present, to establish pure cultures of the streptococcus after at most 12 hours; and cultivations 12 to 18 hours old will suffice to prepare a few doses of vaccine, so that treatment can often be commenced within 36 hours. The doses of vaccine should, if possible, be regulated by the movements of the opsonic index, and the object to be aimed at is the elimination of the negative phase and the production of a series of short positive phases, gradually increasing in duration. But in the absence of this exact guide the temperature and pulse must be carefully watched, for it may be taken as a general, though not invariable, rule that high temperature and rapid feeble pulse coincide with a low index. The dose at first should be 2·5 to 5 millions. With such a dose the negative phase is short, sometimes absent; consequently a fall in temperature usually occurs in about 12 hours. But the succeeding positive phase is also short, and it is often necessary to repeat the dose in 36 to 48 hours. Subsequent doses are generally needed at

[1] *Brit. Med. Journ.*, 1908, ii. 782.

gradually increasing intervals, until convalescence is established.

2. RHEUMATISM AND CHOREA

Ætiology.—The causation of **rheumatic fever,** and of the chronic affections of the joints generally described as rheumatic, is not definitely known.

Many observers have found micro-organisms in the blood and synovial fluid of patients suffering from acute rheumatism. A very large number of different bacteria have been described in this connection—staphylococci, streptococci, diplococci, and bacilli. For practical purposes the question of the microbial origin of the disease is at present associated with the claims of an organism first discovered by Apert and Triboulet (1898), and subsequently investigated by Wassermann, and in this country by Poynton and Paine.

Walker calls the organism the *Micrococcus rheumaticus.* It would be preferable, perhaps, to call it a streptococcus, as it appears to belong to this group.

With regard to the specific nature of this organism, no certain verdict has been pronounced as yet. The opinions of some authorities as to the unity of the streptococci have already been mentioned. On the other hand, it is difficult to believe that so distinct a clinical disease as rheumatism can be due to the same cocci which produce suppuration. If the rheumatic cocci be merely an attenuated form of these, it is curious that suppuration is practically never seen in rheumatic joints, as it might have been expected that the organisms would not infrequently gain increased virulence in susceptible persons. There is no reason to believe that rheumatic subjects are in any way refractory to suppurative lesions. Walker, however, finds that the rheumatic cocci will grow in a filtered culture-fluid in which other streptococci have been grown and have died out. This, which has been alluded to as Marmorek's test for the unity of the streptococci, appears to show that

the *Streptococcus rheumaticus* is distinct from ordinary pyogenic organisms, whilst many observers consider that the organism isolated from the joints is a variety of the pneumococcus.

It must be mentioned that some observers have failed altogether to find micro-organisms in cases of acute rheumatism. Thus McCrae[1] examined 270 cases with practically negative results; and Triboulet,[2] finding organisms in some cases and not in others, considers that the sterile cases represent the true disease, while those due to organisms only simulate rheumatism.

Menzer's serum.—The serum used by Menzer is prepared from streptococci derived from human sources. The effects produced in cases of chronic rheumatism are very remarkable, as, according to Menzer's account,[3] the injection of the serum gives rise to a reaction of an inflammatory nature at the seats of rheumatic lesions (the joints), which is followed by subsequent improvement This peculiar result of the serum would suggest, as was pointed out by Blumenthal, that the serum is not antitoxic or antibacterial, but contains a toxine similar to tuberculin or mallein. The reaction is exactly parallel to that which follows an injection of the old tuberculin in a patient suffering from a tuberculous joint. Menzer, however, holds that his serum is antibacterial, and from what is known of its mode of preparation it should be of this nature. He suggests the explanation that the reaction is due to destruction of the cocci present in the patient and to the resulting rapid absorption of intracellular toxines. Symptoms of constitutional disturbance also accompany the injections of serum in rheumatic patients, viz. rise of temperature, headache, sore throat, and enlargement of lymphatic glands. Cardiac disease, if present, may at first be aggravated by the remedy, but is subsequently improved. The local

[1] *Journ. of the American Med. Assoc.*, Jan. 3, 1903.
[2] *Gazette des Hôp.*, April 4, 1903.
[3] *Zeitschr. f. klin. Med.*, 1902, xlvii. 109.

inflammatory symptoms are not seen in cases of gonorrhœal rheumatism which are treated with the serum.

Menzer gives in his original paper an account of seven cases of rheumatism treated by his serum, and holds that they were all improved. Relapse did not follow the treatment. In one case, which exhibited symptoms of nephritis, these were at first aggravated by the use of serum, but finally improvement was effected; indeed, Menzer suggests that the serum may prove useful in the treatment of some cases of chronic renal disease in which a local stimulating effect may be beneficial. He does not claim that an actual cure of rheumatic fever, or even a cutting-short of the disease, is directly effected by the serum, but thinks that by its means the resistance of the patient is raised. This theory would be consistent with an action analogous to that of tuberculin; which, however, Menzer will not allow.

A patient who had suffered from chronic rheumatism, and had been ineffectually treated by ordinary means, was shown by Menzer at the Berlin Medical Society.[1] As the result of serum-treatment the man had so far improved that he could get about with sticks after four weeks' treatment.

The dose of the serum is not well established. Five to ten cc. may be given experimentally, but large quantities may be used, if no ill effects are observed. Sinnhuber[2] supports Menzer in recommending the use of the serum in cases which have become chronic.

Very great interest attaches to Menzer's experiments, but at present no definite opinion can be expressed as to the merits of the treatment. Cases of acute rheumatism generally yield readily to the use of salicylates, and in view of this fact it is natural to hesitate somewhat before prescribing a remedy which seems capable of giving rise to

[1] *Berlin. Verein f. inn. Med.*, March 23, 1903. See *Centralbl. f. inn. Med.*, 1903, p. 410.

[2] Quoted by Menzer, *Münch. med. Woch.*, 1904, p. 461.

alarming symptoms. It would seem, for the present at least, wise to refrain from using the new remedy in cases complicated by recent endocarditis or other acute lesions of important parts (pleurisy, pericarditis, &c.). In chronic cases, however, it might be safely tried. These are so rebellious to treatment by ordinary means that any method which holds out a prospect of success is to be welcomed. No danger seems to reside in the use of the serum in such instances, while it is easy to suppose that the local reaction might have a curative influence by its stimulating effect on indolent tissues.

Chorea.—The connection between this disease and rheumatism is not exactly ascertained. Many writers consider that chorea is a nervous manifestation of the rheumatic poison, while others see in rheumatism only one of several debilitating diseases which may cause the peculiar condition of the motor centres characteristic of chorea. Bacteriological examination has shown the existence in chorea of organisms similar to those which have been found in acute rheumatism, and some experimenters (Poynton and Paine, Walker) have produced in animals, by injection of these organisms, a condition which they consider to represent the chorea of human beings.

Preobrajensky [1] treated several cases of severe chorea, which he considered to be of an infective type, with a polyvalent antistreptococcic serum. Great improvement ensued, very rapid diminution in the symptoms being observed. Relapses occurred in some of the cases, but renewed use of the serum caused their disappearance.

Chorea, like rheumatism, is a disease in which it is very difficult to make sure of the effects attributable to drugs. Much conflict of opinion has, for instance, been exhibited as to the efficacy of arsenic in this ailment; and other remedies have been vaunted as specifics, only to be rejected in the light of further observation. Hence the claims of antistreptococcic

[1] Quoted in *La Semaine Méd.*, 1902, p. 412.

serum to cut short the disease must be closely scrutinised before they are accepted. It is very desirable that further experiments should be made with this treatment.

3. SCARLATINA

Ætiology.—The causation of scarlatina or scarlet fever is not definitely established. Many observers have described cocci in connection with this disease, and there is a tendency to regard these as the exciting cause of the malady, but so far exact proof is wanting.

Mallory[1] has described "protozoön-like bodies" in the epithelial cells of patients who had died of scarlatina, and is inclined to consider them to be the causal agents. He named the organism (?) provisionally *Cyclaster scarlatinalis*.

In view of the great infectivity of scarlet fever, the contagion being conveyed from one person to another without direct contact, it seems improbable that the *Streptococcus pyogenes* can be the cause of the disease. Conditions due to infection with virulent forms of this organism seem to be propagated only by direct transference (hands, instruments, &c.), and it is unlikely that an attenuated germ, such as that of scarlatina would almost undoubtedly be, could be more readily communicated than the virulent kind. Provisionally, we may conclude that the disease is due to some organism at present unidentified, either owing to its ultra-microscopical size, or to failure to cultivate it outside the body. The constant association of streptococci with scarlatina would indicate that the diseased tissues afford a favourable pabulum for these cocci, which take advantage of the opportunity thus offered, and are probably responsible for many of the complications of the malady, such as ulceration of the throat and disease of the middle ear, just as they appear to excite suppurative lesions in other acute diseases.

[1] *Journ. of Med. Research*, Jan., 1904, p. 483.

SPECIFIC SERUM

Moser,[1] who found that of 99 cases of scarlatina 73 exhibited cocci in their blood, prepared, by injecting horses with these organisms, a specific antiscarlatinal serum. This was tried by Escherich, apparently with good results. He records that the mortality was reduced by its means to 8·9 per cent., whereas in other hospitals at the same time, where the serum was not used, the death-rate amounted to 13·9 per cent. The injection of the serum was followed by fall of temperature and general improvement.

Bokay,[2] Zuppinger[3] and Winocouroff[4] confirm these observations. Mendelsohn[5] on the other hand reports unfavourably, finding the serum quite useless.

Marpmann[6] prepared a "specific" serum by injecting animals with the blood of scarlet-fever patients, with emulsion of their epidermic scales collected in the peeling stage, and with their urine. He prepares a stronger serum for treatment and a weaker for prophylaxis. Campe[7] reports favourably on the value of the serum, which is called "scarlatin."

ANTISTREPTOCOCCIC SERUM

Baginsky[8] tried Marmorek's serum in cases of scarlatina, but found that it did not produce any good effect on the course of the disease. Subsequently he made use of a serum prepared by Aronson, with better results, the mortality from the disease falling from 14 to 11 per cent. The figures

[1] Quoted in *La Semaine Méd.*, 1902, Appendices, p. clviii.

[2] *Jahrb. f. Kinderheilk.*, xii. 428.

[3] *Wien. klin. Woch.*, 1905, No. 44.

[4] *Ibid.*, p. 695. *See also* Egis and Langovoy, *Jahrb. f. Kinderheilk.*, 1907, xvi 514; Eguez, *Rousski Vratch*, 1904, p. 1635; Schick, *Deut. med. Woch.*, 1905, p. 2092.

[5] *Ibid.*, 1905, p. 461.

[6] Abstr. *Centralbl. f. inn. Med.*, 1905, p. 999.

[7] *Berlin. klin. Woch.*, 1905, No. 52.

[8] *Ibid.*, 1902, p. 394.

do not seem very striking. Cumston[1] found a polyvalent antistreptococcic serum useful in "septic" cases of scarlet fever.

In view of the suppurative lesions which may occur as complications of scarlatina (ulceration of the throat, otitis media, &c.), it would seem advisable to use an antistreptococcic serum in severe cases, with a view to prevent or relieve these lesions. It can hardly be expected that the course of the disease itself will be modified by this procedure, but in this country scarlet fever is a mild disease, and it is rather the complications than the primary infection which are to be feared. In the anginose form the use of the serum would seem specially indicated, as this is almost certainly due to secondary infection. The mortality in these cases is high, and even in those which recover the convalescence is long and tedious.[2]

SERUM OF CONVALESCENTS

Leyden[3] has observed the effects of injecting serum derived from convalescents from scarlet fever into patients suffering from the disease. He thinks that good results ensue. According to his observations, the disease is cut short, the temperature reaching normal some days earlier than it would otherwise have done. No critical fall of the fever is, however, seen. The doses administered were from 10 to 20 cc., and no ill effects were produced.

These experiments are of theoretical rather than practical interest, as it is not to be expected that such a remedy could become generally used. Convalescents from a disease could not be expected to sacrifice a portion of their blood for the benefit of other patients—at all events, not in this country. A perusal of Leyden's paper does not convey the impression that the observed results were at all strikingly encouraging.

[1] *Brit. Med. Journ.*, 1908, i. 1290.

[2] Mackie has used antistreptococcic serum in these cases with good results (*Lancet*, 1904, i. 493).

[3] *Deut. Arch. f. klin. Med.*, Bd. lxxiii.

VACCINE-TREATMENT

Gabritchewsky[1] has prepared a vaccine consisting of streptococci isolated from cases of scarlatina, and uses it as a prophylactic. Two injections are given, which are followed by some fever and redness at the seat of injection.

4. PNEUMOCOCCIC INFECTIONS

Causation. — The organism to which the vast majority of all cases of acute pneumonia are due is the *Diplococcus pneumoniæ*, or pneumococcus of Fraenkel and Weichselbaum.

Another organism occasionally associated with pneumonia is the pneumobacillus of Friedlaender (1883). The bacillus of Friedlaender may also give rise to a form of membranous sore throat, and has been found in abscesses in various parts of the body, as well as in pleurisy, endocarditis, otitis, rhinitis, &c. The pneumobacillus is *agglutinated* by the serum of patients infected by it, and exhibits chain-formation if grown in immune serum. A special phenomenon, "amorphous agglutination," the nature of which is doubtful, is described by Schmidt.[2]

Agglutination.—The diplococci are agglutinated by the serum of immunised animals or of patients suffering from the disease. The reaction is not so easily demonstrated as in the case of *B. typhosus*, *Micrococcus melitensis*, &c.; and it does not appear to be of much practical value for clinical diagnosis. The phenomenon can be best shown by growing the cocci in some of the immune serum, a control growth being made in normal serum. In the latter the organisms produce a turbidity of the fluid, and under the microscope are seen to be uniformly scattered about in pairs or short chains. In agglutinative serum the organisms grow in flocculi, while the rest of the fluid remains clear. Micro-

[1] *Bull. Inst. Pasteur*, v. 575 ~~806~~; *Centralbl. f. Bakt.*, 1906, xli. 17, 729. *Cf.* Langovoy, *Ibid.*, 1906, xlii. (*orig.*), 362, 463.

[2] *Münch. med. Woch.*, 1903, No. 30, p. 873.

scopically the cocci are seen to be adherent in long chains or in clumps.[1] Jehle,[2] who apparently used the ordinary technique for agglutination-experiments, reports that in children the reaction appears early in the disease, so as to be of diagnostic value, and that it may be useful for prognosis, since pure pneumococcic affections generally tend to recovery.

SERUM-TREATMENT

G. and F. Klemperer[3] were the first to attempt to treat cases of pneumonia by means of an antagonistic serum. They prepared this from rabbits immunised by injections of pneumococci, using a precipitate from the blood of these animals which they called "antipneumotoxine." In six cases so treated these authors claimed good results. Washbourn immunised a pony, and Parr[4] an ass; the latter treated twenty-two cases with the serum, with two deaths, both in patients who were moribund at the time of admission; he found that doses of 50 cc. were followed by crisis and recovery. Biggs[5] immunised a horse, and found that the serum would protect rabbits against 1,000 lethal doses of the cocci; but he obtained only indecisive results in man.

Pane[6] has prepared a serum from donkeys, and records very favourable results. This serum was capable of protecting laboratory animals against 3,000 lethal doses. Eyre and Washbourn[7] found it effective against four out of five strains of pneumococci. Maragliano,[8] in 1898, stated that "he was more and more convinced of the antitoxic (?) power of Pane's serum, and of its efficacy in pneumonia." Fanoni[9]

[1] *See* Eyre and Washbourn, *Journ. of Pathol.*, v. 13; and Besançon and Griffon, *Ann. de l'Inst. Pasteur*, 1900, p. 449.

[2] *Wien. klin. Woch.*, Aug. 6, 1903, No. 32, p. 917.

[3] *Berl. klin. Woch.*, 1891.

[4] Quoted by Biggs, *infra.*

[5] *Med. News*, 1899, lxxv. 97, 137.

[6] *Riforma Medica*, 1898.

[7] *Brit. Med. Journ.*, Nov. 11, 1899.

[8] Quoted by Fanoni, *Med. Record*, March 10, 1900, p. 431.

[9] *Pediatrics*, May 15, 1900.

is very confident of the value of this preparation. He employs doses of 40 cc. daily, and finds that the temperature is lowered, the general condition of the patient improved, and resolution of the affected lung accelerated. In children especially good results are to be obtained.

Recently Oreste[1] has written in favour of this serum; but Horder and Scofield[2] found it useless in a case of pneumococcic *endocarditis*.

Lambert[3] has immunised horses and treated cases with the serum. He finds that slight reduction of the temperature is effected, but crisis is not induced. The pneumococci may disappear from the blood, if they are present there; in other words, a pneumococcic septicæmia may be prevented. The total effect obtained is, however, small; and Lambert has given up the use of the remedy.

Römer prepared a serum by injecting horses, cattle and goats with different strains of pneumococci and mixing the serums thence obtained. It is favourably reported on by Knauth,[4] Paessler,[5] Tauber,[6] and others, doses of 10 to 30 cc. being given. The value of serum in cases of *ulcus serpens corneæ*, which is often due to a pneumococcic infection, is disputed.[7]

With regard to the properties of a serum prepared by injections of *Diplococcus pneumoniæ* into animals, it would be natural to expect that it would be bactericidal, not antitoxic. On the other hand, it is found that the cocci will grow in the serum of patients suffering from the disease and

[1] *Gaz. degli Osped.*, 1906, No. 22.

[2] *Lancet*, 1905, i. 1333.

[3] *Journ. of American Med. Assoc.*, 1900, i. 901.

[4] *Deut. med. Woch.*, 1905, No. 12.

[5] *Deut. Arch. f. klin. Med.*, Hft. 3 and 4.

[6] *Wien. klin. Woch.*, 1906, No. 11.

[7] *See* Römer, abstr. *Biochem. Centralbl.*, 1903, 66, and Gatti, abstr. *ibid.*, p. 158. Allen isolated a special coccus from cases of ulcerative keratitis and found vaccine-treatment with it useful. The employment of a pneumococcus-vaccine was ineffective. ("Opsonic Method of Treatment," 1908, p. 224.)

in that of immunised animals (*see* p. 238). Bokenham[1] states that the serum has no effect on the cocci by itself: if, however, it is brought in contact with the organisms in the presence of leucocytes, it causes these cells to destroy the germs by phagocytosis, *i.e.* it has an opsonic influence.

SERUM OF CONVALESCENTS

Weisbecker[2] first employed this method of treatment in a series of 21 cases, but in spite of some subjective improvement he considered that the results were inconclusive. Marchoux[3] thought that he observed good effects with this method.

VACCINE-TREATMENT

Vaccine-treatment may prove useful in cases of localised infection by the pneumococcus. Thus Magruder and Webb[4] gave injections of 14 and 20 millions of pneumococci in a case of pneumococcic *otitis media*, with benefit; and Lyon[5] successfully treated a case of *empyema*, which did not heal up after evacuation of the pus, by inoculation of the organisms (100 and 200 millions).

Robinson[6] gave injections of from 11 to 23 millions in a case of pneumococcic *peritonitis* with good effect; and Eyre[7] records cases of pneumococcic *pyæmia* and of *peritonitis* due to the pneumococcus successfully treated by autogenous pneumococcic vaccines, and is convinced of the value of vaccine-treatment in pneumococcic infections.

The present writers recommend quite small doses, viz. 5, 10, and 25 millions, the vaccine being invariably

[1] *Brit. Med. Journ.*, 1900, ii. 1080.
[2] *Münch. med. Woch.*, 1899.
[3] *Ann. de l'Inst. Pasteur*, 1899, p. 193.
[4] *Laryngoscope*, Nov., 1907.
[5] *Lancet*, 1905, i. 1718.
[6] *Brit. Med. Journ.* 1909, i. 651.
[7] Erasmus Wilson Lectures. *Lancet*, 1908, i. 539.

prepared from the organism isolated from the patient. The effect of such a vaccine upon the temperature and general condition of cases of empyema doing badly after operation is often particularly striking, and it would seem that in some cases of lobar pneumonia the crisis is accelerated by some days by the use of vaccines. *Bronchopneumonia* due to the pneumococcus is also amenable to treatment with pneumococcus-vaccine, especially chronic cases in children.

DIPHTHERIAL ANTITOXINE

Talamon[1] has treated 50 cases of pneumonia with antitoxine, many of them being alcoholic patients, and some of them old persons. The death-rate was 14 per cent., whereas previously, in cases treated symptomatically, it had been 37 per cent. He finds that if the serum is administered before the fifth day of the disease, the mortality is only 4 per cent.; whereas, if it is not given till after the sixth day, the death-rate rises to 24 per cent. He gives doses of 20 cc., repeated if necessary; in grave cases he injects 20 cc. at once, and repeats the dose on the following morning and evening. Bessoni[2] also recommends this treatment, reporting 21 cases in which it was used, with a mortality of 4 per cent.; among 79 other patients not so treated the mortality was over 16 per cent. Legros,[3] who made use of antitoxine in some cases of pneumonia, failed to obtain any benefit. The same criticism probably applies to the use of diphtherial antitoxine in pneumonia as in septic conditions (*see* p. 228).

O'Malley[4] believes that antitoxine is a very valuable therapeutic agent in cases of *bronchopneumonia* in children, especially in the forms which complicate other infective diseases (measles, influenza, &c.).

[1] *La Semaine Méd.*, 1901, p. 69.
[2] *Ann. de Méd. et Chir. Infantiles*, Feb. 15, 1899.
[3] *La Semaine Méd.*, 1901, p. 158.
[4] *American Medicine*, Jan. 17, 1903.

SUMMARY

The results obtained up to the present with anti-pneumococcic serum are disappointing. It is certain that good effects are produced in animals, but these are in the direction rather of prophylaxis than of cure. In man, by the time that symptoms of pneumonia have developed the cocci have gained so firm a footing, and have increased to such numbers, that only a very potent serum of a bactericidal nature could be expected to act efficiently. Those which are at present obtainable do not seem to come up to this standard. It is also possible that the immune bodies formed in the lower animals do not find suitable complements in man. Further, it appears that there are different strains of the pneumococcus which react differently to antibodies. More might be expected from an antitoxic than from a germicidal serum, as pneumonia is characterised by symptoms of profound intoxication; but as it has not been possible to obtain potent solutions of the toxines of the *Diplococcus pneumoniæ*, no such serum is at present available, nor is there any immediate prospect of its preparation. Vaccine-treatment is still in its infancy, but already gives promise of future success.

5. AFFECTIONS DUE TO THE GONOCOCCUS

Diplococcus gonorrhœæ, or gonococcus, was first described by Neisser in 1879. It usually gives rise to a local suppurative affection (urethritis, conjunctivitis), but it may cause a general infection, the organisms entering the circulation and inducing arthritis, endocarditis, and septicæmia.

Toxines and antitoxines.—Christmas [1] has grown the cocci artificially and obtained a poisonous fluid. A toxic solid, gonotoxine, can be precipitated from this by ammonium sulphate; it is not dialysable, and is not destroyed by heating to 65°C. for half-an-hour. By injection

[1] *Ann. de l'Inst. Pasteur*, 1900, p. 331.

of the toxine into rabbits an antitoxic serum can be prepared, which neutralises the poison *in vitro*, and acts prophylactically against injections of the poison in animals. It does not seem to have been used on man.

Rogers and Torrey [1] prepared a serum by injecting rams first with heated and then with fresh cultures of the gonococcus. They found it useless as a remedy in urethritis and conjunctivitis—localised lesions—but of some value in gonorrhœal septicæmia. Uhle and Mackinney [2] also found serum-treatment useful in gonorrhœal arthritis, but ineffective against the urethritis caused by this organism.

VACCINE-TREATMENT

In cases of gonococcal urethritis of a subacute type the opsonic index is usually low (0·6); in those which rapidly convalesce it is high (1·8), whilst those which merge into an intractable gleet vary almost from day to day, within wide limits. Vaccine-treatment is of value in all stages, whilst in severe cases of gonorrhœal sapræmia or septicæmia with pyrexia and prostration the effect of vaccines is most marked.

Eyre and Stewart [3] advise that, whenever possible, autogenous vaccines should be employed; but if such a course is impossible, polyvalent stock vaccines, prepared from five or ten different strains of gonococci, must be used. The initial doses should not exceed five millions. With this dose the negative phase is, as a rule, short, and is marked clinically by exacerbation of the discharge, lasting from one to three days, and followed, as the positive phase becomes established, by a rapid diminution. After a second injection this sequence of events is repeated. Urethral irrigation should at first be forbidden. Later, when the patient has shown a satisfactory response to the inoculations, the opsonic index should be estimated daily, until the

[1] *Journ. Amer. Med. Assoc.*, 1907, xlix., No. 11.
[2] *Ibid.*, July 11, 1908.
[3] *Lancet*, 1909, ii. 76.

positive phase is well established at, say, 1·4 or 1·8. At this point irrigation of the urethra with some simple aseptic solution, such as potassium permanganate, 1 in 500, should be instituted, and it will usually be found that after a few days the discharge entirely ceases.

One point of considerable importance must be insisted upon—namely, that cases of chronic gleet of many years' standing are frequently not benefited by gonococcus-vaccine. The gonococcus in such cases appears to have died out, and various staphylococci, often of very low virulence, are now responsible for the persistence of the "morning drop," and the administration of a vaccine prepared from such staphylococci rapidly results in a cure.

Eyre and Stewart[1] have summarised the vaccine-treatment of gonococcus injections as follows—

"*I. Acute gonorrhœa.*—1. Gonococcus-vaccine is markedly toxic and exerts a profound influence over the disease.

"2. For routine work (hospital out-patients, &c.) vaccine-treatment is not devoid of danger and requires the exercise of considerable caution.

"3. A stock vaccine, comprising a dozen different strains, gives results only slightly inferior to those observed when using a vaccine prepared from the patient's own organism. This is not the rule in most other diseases.

"4. Small doses, repeated at short intervals, are more effective than large doses at lengthened intervals.

"5. Small doses of vaccine (from 1,000,000 to 10,000,000 cocci) are safer and more satisfactory than the large doses (from 50,000,000 to 100,000,000) which are often prescribed.

"6. After an injection of from 500,000 to 2,000,000 the negative phase is either absent or extremely transient.

"7. An inoculation of from 5,000,000 to 10,000,000 causes a negative phase of usually not longer than 48 hours' duration, followed by a positive phase of from three to five days.

"8. Vaccine in *small* doses serves the double purpose of *raising and steadying* the opsonic index. A steady index just above normal is found to be the most favourable condition for rapid recovery.

"*II. Simple chronic gonorrhœa.*—1. Where the gonococcus has ceased to be the infecting organism, these cases are on a par with

[1] *Op. cit.*

other chronic inflammatory states, but are frequently more difficult to cure owing to environment and local conditions.

"2. Chronic cases, where the gonococcus is the sole infecting organism, have a better prognosis from the point of view of treatment by vaccine than a mixed infection or one of staphylococcus only.

"*III. Chronic gonorrhœa with complications.*—1. The estimation of the opsonic index is helpful to diagnosis, and is a useful means of determining *approximately* the opsonic state of the blood. Chronic gonococcus infections, however, present clinical features which themselves afford valuable indications during the course of vaccine-treatment.

"2. Where the gonococcus alone is the infecting organism, if the opsonic index cannot be obtained as frequently as is desirable, routine injections of from 1,000,000 to 2,000,000 cocci every three to five days, are safe and satisfactory; a lapse of five to seven days after doses of 5,000,000; an interval of eight to ten days after inoculation of 10,000,000. Larger doses than these are seldom desirable.

"3. Treatment by small and gradually increasing doses at frequent intervals should at all times be observed: the use of large doses is even more dangerous than in acute cases, and may be followed by disastrous consequences.

"4. In orchitis small doses of vaccine quickly relieve pain and cause a more rapid abatement of symptoms than is obtained by the usual routine treatment alone.

"5. In iritis the severe pain, which is a marked and obstinate feature, is relieved in 48 hours after an injection, and disappears in from three to four days; cure is much hastened.

"6. In arthritis the treatment is of considerable value."

6. CEREBRO-SPINAL MENINGITIS

This affection is due to the *Meningococcus* or *Diplococcus intracellularis meningitidis*, discovered by Weichselbaum. Little is known of the toxines of this organism, which have not been obtained in culture-media. Serum for therapeutic purposes has been prepared by injecting animals with the cocci themselves, and is presumably bactericidal, not antitoxic.

Jochmann[1] obtained from horses, goats and sheep a serum which agglutinated the cocci in dilutions of 1 : 300 to 1 : 15,000. He employed it on human patients, both

[1] *Deut. med. Woch.*, 1906, No. 20.

subcutaneously and by lumbar puncture, with good effects. Raczynski,[1] however, failed to observe any benefit from the use of this serum.

Flexner and Jobling[2] also prepared a serum which was used in 421 cases with a mortality of 33 per cent., or, excluding moribund cases, of 25 per cent. Robb[3] treated 90 cases with the serum, with a mortality of 30 per cent.

A serum prepared by Kolle and Wassermann has been used in Germany, Switzerland and Italy. Többen[4] found that the mortality in his cases fell from 56·7 per cent. to 34·5 per cent.; and Krohne[5] similarly reduced the mortality from 66 to 47·6 per cent., the mortality in cases treated within the first two days being 33 per cent.

Currie and MacGregor[6] treated 105 cases with serum, using several different brands, with a mortality of 64·8 per cent., as compared with a mortality of 79·5 among 225 cases not so treated: the difference is not very striking. They collected from other sources the following statistical results in addition to those already mentioned:—Wassermann, 57 cases, deaths 47·3 per cent.; Jochmann, 17 cases, deaths 29 per cent.; Robb (1908), 30 cases, deaths 26·6 per cent.; Dunn, 15 cases, deaths 20 per cent.; and Levy, 40 cases, deaths 11·76 per cent. The chief inference from the figures would appear to be that the disease varies much in severity at different times and in different places. Ker[7] treated 33 cases with Flexner and Jobling's serum, with a mortality of 40 per cent. and believes that the remedy shortens convalescence.

Mackenzie and Martin[8] used the *serum of convalescents*,

[1] *Wien. klin. Woch.*, 1907, No. 52.
[2] Stud. from Rockfeller Inst., Reprints, 1909.
[3] *Lancet*, 1907, i. 1213.
[4] *Münch. med. Woch.*, 1907, p. 2420.
[5] *Zeitschr. f. Medizinalbeamte*, 1908, No. 78.
[6] *Lancet*, 1908, ii. 1072.
[7] *Edin. Med. Journ.*, Oct., 1908, p. 306.
[8] *Lancet*, 1908, ii. 477.

with a mortality of 37·5 per cent., and *diphtherial antitoxic* serum was tried by Huber,[1] Waitzfelder,[2] and Balduzzi,[3] with apparent benefit.

Elder and Ievers[4] used injections of Rienzi's *anti-pneumococcic serum* in cases of cerebro-spinal meningitis, on the ground of the relationship between the organism to which this affection is due and the pneumococcus, and thought that some improvement was produced.

In order to be effective in this disease the serum must be injected into the spinal canal by lumbar puncture: given subcutaneously, it is quite useless.

Agglutination.—The agglutination-reaction occurs in most cases of cerebro-spinal meningitis, and may be used as a test for diagnostic purposes. It may, however, be absent during the first few days of the disease.[5]

Young cultivations of a strain of meningococcus that has been frequently sub-cultivated in the laboratory should be used for this reaction.

VACCINE-TREATMENT

Hector Mackenzie[6] treated a case with injections of a vaccine consisting of dead meningococci. The first dose consisted of 120,000,000 cocci, followed 10 days later by 20,000,000, and again a week later by 143,000,000. A final injection of 71,000,000 organisms was given a week later. The vaccine was prepared from organisms grown from the patient's cerebro-spinal fluid, obtained by lumbar puncture. The patient recovered. Rendle and Mottram[7] also made use of a vaccine of dead meningococci in doses of 200,000 and 500,000 organisms, and claim that benefit

[1] *New York Med. News*, Apr. 16, 1905.
[2] *Med. Record*, Mar. 11, 1905.
[3] *Gaz. degli Osped.*, 1907, No. 36.
[4] *Scottish Med. and Surg. Journ.*, 1907, xx. 215.
[5] Houston and Rankin, *Lancet*, 1907, i. 1213; 1908, ii. 474.
[6] *Brit. Med. Journ.*, 1907, i. 1408.
[7] *Lancet*, 1907, ii. 220.

resulted from the treatment. We have treated three cases with vaccine, but without averting the ultimate fatal issue.

The *opsonic index* of the blood is usually low (0·4) in cases of cerebro-spinal meningitis, and may be raised by injections of suitable vaccine, but owing to the localisation of the meningococci, vaccine-treatment is not to be preferred to serum-therapeutics. The estimation of the opsonic index is, however, occasionally of value as an aid to diagnosis.

CHAPTER XIII

TUBERCULOSIS

Causal bacillus and toxines.—The *Bacillus tuberculosis* was discovered by Koch in 1882. By growing the bacteria in glycerine-bouillon some of the toxines may be obtained, but the bodies of the bacilli are themselves toxic, acting as distinct irritants even when they are dead. The toxines of the tubercle-bacillus are not well understood. A peculiar acid formed by it has been credited with the power of producing the characteristic degeneration of the tissues called "caseation." Another substance gives rise to fever; and certain volatile products are said to have a convulsant effect. The bacilli may cause death in two ways—either by rapid multiplication and formation of toxines, as in acute miliary tuberculosis, or by producing gradual destruction of some organ necessary to life, such as the kidney or the lung. In these latter cases there is often a superadded infection by pyogenic organisms, staphylococci, &c., to the toxic effects of which the profuse sweats so characteristic of advanced tuberculosis are principally due, and very possibly also much of the ulcerative destruction of tissue met with in cavities in the lungs as well as in superficial lesions.

The question of the identity or difference of human and bovine bacilli is still undecided. Professor Koch holds that the organisms are distinct, and that bovine tubercle cannot be conveyed to man, nor human tubercle to cattle. The general opinion at the present time appears to be that there is no such absolute distinction between them, although the difficulty of infecting bovine animals with human bacilli

is admitted to be very great. Birds suffer from a special form of tubercle, which appears to be caused by a distinct, though nearly allied, organism. Use has been made of the different varieties of the *B. tuberculosis* for the purpose of immunising animals, inoculation with bacteria of the kind special to another animal not producing tuberculosis, but rendering the animal inoculated immune against its own form of the disease.

TUBERCULIN

Artificial preparation of the toxines of the tubercle bacillus. Koch found that by growing the bacilli from six weeks to two months in flasks containing slightly alkaline veal broth, to which a percentage of peptone and of glycerine had been added, and freely supplying the cultures with oxygen throughout, it was possible to obtain a fluid containing some, at any rate, of the toxines produced by the organisms. By passing this through a porcelain filter the bodies of the bacteria were removed, and a solution of the poisons remained. This was concentrated by evaporation to one tenth of its bulk, and to the fluid thus obtained the name of *tuberculin* was given. It was hoped that this preparation would exert a curative effect on the disease (phthisis), and the discovery was announced to the world as a "cure for consumption." Great hopes were thus raised, only to be dashed to the ground when further experience was gained as to the limitations of the method and the inconstancy of the good results produced. The reaction which ensued as the result of this disappointment undoubtedly went too far in the opposite direction, and the really valuable properties of tuberculin were overlooked or denied.

Composition of tuberculin.—Tuberculin, as thus prepared, is a somewhat thick fluid, of dark-yellow colour. It is practically a solution in glycerine of the toxines formed by the bacillus, since the glycerine added to the original culture-medium does not evaporate on heating, while the

water is driven off. A careful analysis of tuberculin, as originally prepared by Koch, was made by W. Hunter,[1] as the result of which he came to the conclusion that it was a very complex substance. He separated from it (besides glycerine, &c.) alkaloids, albumoses, and extractives. He considered that the material which produces the febrile reaction was of the last class, and as these matters are separable by dialysis, he hoped to produce a tuberculin free from the objectionable features of Koch's preparation. The remedial substance is probably an albumose, as is also that producing the inflammatory reaction around the foci of tuberculosis. There are thus at least three active principles present in tuberculin.

Koch, as the result of independent analysis, agreed in considering that the most important material was of the nature of an albumose, but he was doubtful as to its exact chemical nature, owing to its power of resisting high temperatures—a peculiarity which Hunter also had noted.

Modifications of tuberculin.—Since the original announcement of the remedy, modifications in the mode of preparing tuberculin have been introduced by Koch.[2] (1) Instead of the toxine produced by growth of the bacilli in a fluid medium, he has made use of extracts of the organisms themselves. He took highly virulent cultures of tubercle-bacilli, dried them *in vacuo*, and triturated them in a mortar. The resulting powder was treated with sterile distilled water, and submitted to centrifugalisation. The supernatant clear but opalescent fluid was then removed from the *débris*, and 20 per cent. of glycerine added as a preservative. To it Koch gave the name of Tuberculin-O (T.O.), or "*Oberer* (upper) Tuberculin." This corresponds in composition and action with the original tuberculin T.O.A. (Tuberculin original alt). (2) The solid residue thus freed from soluble toxines was then dried, and the process of extraction by triturating with 20 per

[1] *Brit. Med. Journ.*, 1891, ii. 169.

[2] *Deut. med. Woch.*, 1897, p. 209.

cent. glycerine-solution and then centrifugalising was repeated several times, the fluid used each time being preserved, and the whole finally mixed together. This mixture constituted the New or Residual Tuberculin (*T. rückstand*, T.R.). Koch claims that specially valuable properties reside in this last preparation, gradually-increasing doses injected into animals producing immunity to tuberculosis, and also to the action of the other forms of tuberculin. (3) A third modification (T.A., *Tuberculin alkalinum*) is obtained by extracting dried tubercle-bacilli with decinormal soda-solution, and filtering the fluid. Tuberculin-O and Tuberculin-A produce effects very similar to those of the old tuberculin (T.). (4) A fourth modification known as "New-Tuberculin B.E." (bacillus emulsion) is merely a suspension of 5 milligrammes of powdered tubercle-bacilli in 1 cc. of 50-per-cent. solution of glycerine.

All the preparations previously mentioned are manufactured from *B. tuberculosis* of the human type. Precisely similar preparations are made from *B. tuberculosis* of the bovine type, and these are indicated by prefixing the letter P. (Perlsucht) to the letter or combination of letters which stand for those prepared from human tubercle-bacilli.

TUBERCULOCIDIN AND ANTIPHTHISIN

It has been suggested that in the process of preparing tuberculin usually adopted, some of the constituents of the bacterial culture, which are volatile, are driven off by the heat. Tuberculin also contains some ingredients which are harmful rather than curative in nature. Klebs has therefore produced modifications of tuberculin, prepared without heating, to which he has given the names of "tuberculocidin" and "antiphthisin." The method of manufacturing the former is complicated, consisting in frequent precipitation, and solution of the precipitate, by alcohol and other reagents. Tuberculocidin is said to kill the bacilli when it is added to a culture of them *in vitro*.

Klebs[1] claims good results from the use of his preparation, and a large amount of antiphthisin is said to be used in America. The therapeutic dose of tuberculocidin is 1 centigramme to start with, the fluid as sold being diluted before use (1 : 10). Antiphthisin is more concentrated, and half the above quantity is administered.

Tuberculocidin may also be administered by the mouth, and is said to act as well or even better by this channel. It may be applied locally to tubercular lesions, and forms an efficient dressing for ocular ulcers, tuberculides, &c. Disease of bones and joints also improves under treatment with this remedy; in cases of tubercular hip-disease it should be injected in the neighbourhood of the joint. Tuberculocidin is said to have a favourable influence in cases of genito-urinary tuberculosis.

Jessen[2] states that the remedy is not free from danger. He gives doses of 20 to 25 drops, finding that larger doses may produce a reaction like that seen with the old tuberculin. He reports good effects from the use of tuberculocidin in phthisis—fall of temperature, diminution in the amount of sputum, and feeling of improvement. All his cases in the first stage of the disease showed improvement (100 per cent.), and 54 per cent. of those in the second stage. Jessen thinks the remedy worthy of further trial.

There does not seem to be sufficient evidence available as to the curative effects of these preparations. Klebs claims 60 per cent. of cures among his cases, which does not seem a startling figure.

BOVINE TUBERCULIN

Spengler and Raw[3] have advised the use of a tuberculin derived from cultures of bovine bacilli ("Perlsucht Tuberculin": P.T.O.), either in place of the ordinary preparation or in conjunction with it (*vide supra*).

[1] *Münch. med. Woch.*, 1904, p. 1688.
[2] *Centralbl. f. inn. Med.*, 1902, No. 23, p. 585.
[3] *Deut. med. Woch.*, 1908, No. 38.

OXYTUBERCULIN

Hirschfelder[1] believed that toxines were converted into antitoxines by a process of oxydation, and consequently prepared a modification of tuberculin, which he called "oxy-tuberculin," by treating the ordinary substance with hydrogen peroxide. The hypothesis upon which the procedure is founded seems quite unsupported by evidence, and no records of the employment of the remedy are available.

VON RUCK'S TUBERCULIN

This is a watery solution or extract[2] of tubercle-bacilli, comparable with Koch's *Oberer Tuberculin* (T.O.). It has been used in the United States with good results. (p. 278).

TUBERCULOPLASMIN

Hahn,[3] by squeezing the bacilli in a hydraulic press, according to Buchner's method, prepared a fluid to which he gave the name of "tuberculoplasmin." We have not been able to find any records of its employment in therapeutics.

TUBERCULOTOXOIDIN (T.B.K.)

Ishigama[4] gave this name to a solution of tubercle-bacilli in sulphuric acid, which he found to have some immunising value and to raise the opsonin-index. He also makes use of a serum prepared from animals immunised with this substance ("tuberculo-toxoidin immune serum").

DENYS' TUBERCULIN

This is prepared in a manner similar to Koch's, but without the use of heat. It is sent out in eight different strengths, named respectively $\frac{\text{To}}{10{,}000}$, $\frac{\text{To}}{1{,}000}$, $\frac{\text{To}}{100}$, $\frac{\text{To}}{10}$, To,

[1] *Deut. med. Woch.*, 1897; *Therap. Beilage*, 25.
[2] *Therapeutic Gaz.*, 1896, p. 308.
[3] Quoted by Shaw, *Lancet*, 1908, i. 926.
[4] Ishigama and Matsuda, Abstr. *Centralbl. f. Bakt.*, 1908, xli. 100.

Ti, Tii, Tiii, each being 10 times the strength of that preceding it, so that a gradual increase of dose is facilitated.[1]

BACILLOSINE

Levet[2] grows the bacilli for three months and then distils the cultures. It is not clear from his account whether he then uses the distillate or the residue for immunising purposes—presumably the former. The preparation is made in several strengths—No. 0, 1 per cent.; No. 1, 2 per cent., and No. 2, 3 per cent. It may be used as a prophylactic.

IMMUN KÖRPER (I.K.)

Spengler[3] has recently devised another immunising preparation to which he gives the name "I.K." (Immun Körper). It is prepared from the red corpuscles of immunised animals, in which he believes the protective substances to be formed, and is administered hypodermically or by rubbing into the skin. It produces, according to its inventor's statement, improvement in breathing and in subjective symptoms, and a disappearance of bacilli from the sputum. It can also be used for diagnostic purposes, as it produces a local reaction around tubercular lesions.

TUBERAL

A remedy sold under this name is said to be an albuminous substance (tuberculo-albumin) derived from the bodies of tubercle-bacilli. It is a greyish-white substance, and for use is dissolved in a 0·3 per cent. solution of phenol. It is administered by the mouth in doses of 10 to 40 drops. We have not been able to find the article in which it was originally described by its inventor, Thamm.

[1] *Bull. R. Acad. Méd. Belg.*, xvi. 499; *see also* Sahli, *Ueber Tuberkulin-behandlung*, 1907.

[2] *Arch. gen. de Méd.*, 1906, cxcvii. 965.

[3] *Deut. med. Woch.*, 1908, No. 38.

TUBERCULIN CL. (CALMETTE)

Calmette prepares a special form of tuberculin, which was originally intended for the ophthalmic test, but which has recently been used for treatment. Broth-cultures, six weeks old, of bovine tubercle-bacilli, are heated in the autoclave at 110° C. for twenty minutes. They are then evaporated on a water-bath at 80° C. to one-tenth of their original volume, and filtered. The filtrate is precipitated with 95 % alcohol, and dried *in vacuo*; it is then dissolved again in water, once more precipitated with alcohol, and dried. The resulting powder is dissolved in sterile water for use, and is injected subcutaneously in graduated doses, from 0·001 mg. to 1 mg., at intervals of twelve days.

BERANECK'S TUBERCULIN

Beraneck [1] has prepared a form of tuberculin (T.B.K.) which he considers to be less toxic than Koch's. He obtains a toxine (*basitoxine*, B.T.) by growing the bacilli in veal-broth rendered slightly alkaline with calcium hydrate, at a temperature of 37 to 38° C., and evaporating the fluid *in vacuo* without heating. This is mixed with an equal quantity of a second toxine (*acidotoxine*, A.T.) extracted from the bodies of the bacilli. The preparation is sent out in a series of different dilutions—$\frac{A}{32}$, $\frac{A}{16}$, $\frac{A}{8}$, $\frac{A}{4}$, $\frac{A}{2}$, A, B, C, D, E, F, G, H, the last being the pure tuberculin, and each of the former brands being twice the strength of that preceding it.

Sahli [2] has used the preparation with good effect, and finds the series of dilutions convenient. He starts with $\frac{1}{20}$ cc. of the dilution $\frac{A}{32}$, and increases the dose, if no result ensues, by successive amounts of $\frac{1}{20}$ cc.; he then uses the stronger solutions successively. The injections are given twice a week, or less frequently.

[1] See *Semaine méd.*, 1903, p. 393.

[2] *Op. supra cit.*

Good results in cases of pulmonary tuberculosis were also obtained by Paris,[1] who used slightly different solutions.

TULASE (Tuberculase)

Recently Behring announced the preparation of an extract of tubercle-bacilli (tulase) from which toxic matters have been removed and which he believes will be useful for treatment and prophylaxis. The exact manner in which the substance is prepared has not been published, and so far the remedy is not generally procurable. Collin,[2] who used some of it in certain cases of ophthalmic tuberculosis, speaks of it as a wax-like residue which separates from the bacilli when they are rubbed up with chloral hydrate and left to stand. The doses given were 0·01 mg. to 8 mg. An immune serum or *antitulase*, prepared by injecting animals with tulase, may also be used to induce a passive immunity.

EMULSION OF BACILLI

Koch,[3] as we have seen, (p. 253) modified his procedure in the matter of inoculation in tuberculosis, and preferred to use, instead of the toxines of the bacilli or an extract of their body-substance, the actual bacilli themselves. Powdered tubercle-bacilli are suspended in 50-per-cent. solution of glycerine, and the fluid is allowed to stand till all particles of any appreciable size have sunk to the bottom. The supernatant emulsion is poured off, and is ready for use. The quantities are adjusted so that 1 cubic centimetre of the fluid contains 5 milligrammes of powdered bacilli. For use it is diluted with normal solution of sodium chloride. Koch starts with subcutaneous injections calculated to contain 0·0025 mg. of the solid material. He repeats the dose every two days or so, raising the quantity administered each time to twice, or even five times, the amount of the previous dose. No

[1] *Rev. Méd. de la Suisse Romande*, 1904, No. 10, p. 629.
[2] *Münch. med. Woch.*, 1907, No. 36.
[3] *Deut. med. Woch.*, Nov. 25, 1901.

reaction occurs as a rule with the first small doses. It may occur as the dose is raised, and when it is observed the intervals between the injections must be prolonged (eight days or so).

The agglutinative power of the serum in patients so treated rises rapidly in the great majority of instances. In a minority it remains stationary, or may even sink. In such cases Koch gives intravenous injections of a fluid corresponding with his earlier T.O. (*see* p. 252), but only very small doses can be administered in this way. Koch considers, however, that the intravenous method has great advantages; and since introducing it, he has in many cases started with the subcutaneous mode of injection and had recourse to the intravenous method afterwards, as soon as a reaction occurred with the former.

Koch finds that with this treatment the patients gain appetite and weight. Night-sweats cease, moist sounds disappear from the lungs, and the amount of sputum is reduced. The presence of fever is not a contra-indication to this treatment; indeed, pyrexia is reduced by it.

Action of tuberculin.—It is found that if a minute quantity of the original preparation (T.O.A.) is injected hypodermically into a patient or animal suffering from tuberculosis, very definite symptoms are produced. There is a rise of temperature of varying intensity, from one to three or more degrees Fahrenheit, accompanied by a feeling of illness and sometimes by nausea or even vomiting. At the seat of any localised focus of tuberculosis which is open to observation, there occurs a more or less vigorous reaction, with heat and redness; and often, if this is severe, there is a casting-off of necrosed pieces of tissue. After the reaction has subsided, it is seen in many cases that an improvement in the local disease has set in, with a tendency to healing in what may have previously been an indolent sore. The tubercular disease of the skin, known as lupus vulgaris, is the form of tubercle in which this result is best seen, but

the same phenomena may take place in any focus of the disease.

The mode of production of the fever, in the case alike of the old and of the new tuberculin, is not well understood. It cannot be due to the existence in the tuberculin of a direct thermogenic substance, as in that case normal individuals would be affected in the same way as the tuberculous. It has been suggested that in the case of the old tuberculin the fever is the result of the local inflammation excited around the lesions, but this is doubtful in view of the similar action of the new preparation, which is not followed by any such local effects. We are driven to suppose that there is an interaction between two substances, one contained in the tuberculin and the other present in the body of the tuberculous individual, the result of which is the formation of some pyogenic substance as yet unknown.[1] The explanation put forward by Ehrlich is as follows:—The normal cells of the body are not affected by this substance, nor are those which form the actual tubercular tissue. Probably the latter are habituated to the poison, as they are in close relation with the bacilli which are constantly giving rise to its formation. There is, however, a zone of cells at a certain distance from the centre of infection which have been only so far affected by the poisons of the bacillus as to be rendered unusually susceptible to their influence. When an injection of tuberculin is administered, an additional quantity of poison is brought into contact with these cells, and they are thus stimulated to react. The reaction takes the form of inflammation—the process by which dead or dying tissues are cast off from the body, as is seen in the separation of a sequestrum or a slough. Hence the necrotic tubercular tissues tend to be cast off by the action of the tuberculin, and a more or less healthy granulating surface is left in favourable cases.

[1] Krehl and Matthes found that albumoses derived from many different sources produced effects precisely similar to those of tuberculin. (*Arch. f. exper. Path. u. Pharmak.*, 1895, xxxvi. 437.)

The action of the new tuberculin (T.R.) is quite different, as far as can be observed. The injection of a small quantity of this—the actual substance of the bacteria—causes, indeed, a general reaction of a febrile nature in tuberculous patients, but this is unconnected, as far as can be seen, with changes at the site of existing lesions. The curative effect of the injections is exerted by stimulating the tissues of the body generally to form antibodies to the tubercle-bacilli. In other words, the new tuberculin is supposed to give rise to a condition of general immunity. It certainly seems to have the power of raising the agglutinating power of the serum, and also its opsonic properties, and there is reason to believe that these increase *pari passu* with its antibacterial properties.

Marmorek maintains that an interaction takes place between the tubercle-bacilli themselves and the tuberculin; and states that a febrile reaction may occur if tuberculin is injected almost immediately after inoculation of bacilli. This statement, however, is not confirmed by other observers.

Patients in the earlier stages of tuberculosis appear to react to tuberculin more strongly than those in whom the disease is more advanced; indeed, those in the third stage of phthisis may fail to give any reaction. This may be due to the fact that their tissues have become habituated to the toxines. It does not detract from the practical diagnostic value of the drug, since it is in the early stages especially that the disease is difficult to recognise.

The anatomical effects produced in a tuberculous subject by an injection of tuberculin may be best seen in an infected guinea-pig, which has been killed by the injection of a large dose of this substance. A zone of hyperæmia may be seen surrounding each of the grey nodules characteristic of the disease, which occur throughout all the internal organs. Healthy guinea-pigs can tolerate even large doses of tuberculin without manifesting any symptoms; tuberculous animals are killed by a moderate dose. Human beings are

apparently more susceptible to tuberculin than are guinea-pigs. These facts tend to show that at all events a possible source of danger resides in this substance when it is used on tuberculous patients. They also seem to prove that, as is the case with the fever-producing substance noted above, tuberculin does not contain a substance directly poisonous in itself, but rather that it contains some material which interacts with another substance present in infected individuals, the two together forming a poison. The reaction would seem to depend to some extent on the establishment of a state of "anaphylaxis."

By-effects of tuberculin.—The injection of the original tuberculin (T.O.A.) may be followed by the appearance of a rigor in some instances, and albumin may be found in the urine. Pains in the joints may occur, as after injection of serum. In some cases jaundice has resulted, and affections of the skin may be produced. Thus, purpuric eruptions have been recorded, and Thin [1] quotes an instance in which a generalised scarlatinal rash appeared, followed by desquamation.

The new tuberculin (T.R.) may also produce rigors, and severe headache may occur after an injection. Albuminuria is also met with, and may be considerable in amount.[2]

Cranston Low [3] records the appearance of a rash resembling lichen scrofulosorum. Weischer [4] saw an acute pleurisy arise, apparently as a consequence of an injection of tuberculin, and we have seen a similar occurrence as the result of too large a dose.

In a few instances death has followed an injection, and has been attributed to the action of the tuberculin.[5]

The existence of nephritis, other than tuberculous, is a contra indication to the use of either variety of tuberculin.

[1] *Brit. Med. Journ.*, 1890, ii. 1330.
[2] Adrian, *Arch. f. Derm. u. Syph.*, 1898, Bd. 45, p. 97.
[3] *Scott. Med. and Surg. Journ.*, Sept., 1905.
[4] *Zeitschr. f. Tuberk.*, 1905, Bd. vii., Hft. 3.
[5] *See* Adler, *Prag. med. Woch.*, 1904, No. 30, p. 389.

DIAGNOSTIC USE OF TUBERCULIN

Veterinary use.—As a means of recognising the presence of tuberculosis, tuberculin has proved of the greatest service, especially in veterinary practice. It is of considerable importance to be able to discover the existence of the disease in herds of cattle; and by injecting the animals with Koch's preparation the diagnosis can be made with almost entire certainty, even in the absence of any symptoms of the malady. The injection does no harm to the beasts beyond the temporary febrile symptoms which it produces in those which are tuberculous. No effects at all are produced in healthy animals. Thus McEachran[1] records the use of tuberculin in 22,023 cases in cattle without any ill effects.

Employment in man.—In mankind the use of tuberculin as a diagnostic agent has been much debated. That it is of service in this respect cannot be denied, but it has been held that there are *drawbacks* which counterbalance its usefulness.

(1) It is urged that in a certain proportion of cases the injection of tuberculin may light up again an infection which has become quiescent, and may thus cause an exacerbation of the disease. It is very difficult to make certain of the facts in this respect, since tuberculosis is a disease which is very liable to sudden exacerbations without the administration of any drug, and it is probable that many of the ill effects attributed to the action of tuberculin have been only accidental concomitants.

Koch, writing in 1897, remarks:—"The most valuable property of tuberculin is that, even when injected subcutaneously in very minute doses, it gives rise to the characteristic reaction in both men and animals affected with tuberculosis. The value of tuberculin as a diagnostic agent, on which I laid stress in my first publication on tuberculin, has been more and more fully vindicated with the lapse of time. The fear that along with the reaction

[1] *Trans. British Congress of Tuberculosis*, iv. 114.

tubercle-bacilli might be set free and gain a footing in healthy parts of the body has been proved to be unfounded in many thousands of injections into cattle made for the purpose mentioned. In not one single case was it possible to detect any indication of such unfettering of the bacilli. In view of this evidence the foolish prejudice resting on the supposed setting-free of the bacilli should be abandoned, and use should be made of the diagnostic properties of tuberculin." At the British Congress of Tuberculosis Koch quoted 3,000 tests made with tuberculin in man without any ill effects; and Anders [1] alludes to 3,638 similar injections which were equally harmless On the other hand, Munzer [2] and Behring [3] regard the injections as distinctly dangerous; and we believe that, if large doses are given, there is solid ground for such fears.

(2) There is some danger of actually inoculating living and virulent tubercle-bacilli in the tuberculin. This can hardly be the case with the old tuberculin, which is generally used for diagnostic purposes; but in the new tuberculin Thellung [4] found virulent bacilli, and actually produced infection in rabbits and guinea-pigs.

(3) The test cannot be used in cases in which the patient's temperature rises (apart from the use of tuberculin) to as high a point as 100 F., because in such instances it is not possible to make sure of the reaction. It is also apparently unwise to use the drug in febrile cases, as they may be injuriously affected by it.

(4) Most important of all as a drawback to the use of the test for diagnosis is the fact, now ascertained, that the reaction is obtained not only in cases of active tuberculosis, but also in old quiescent cases (and it is in these that there appears to be some danger of lighting up the disease afresh),

[1] *Trans. Amer. Climatol. Assoc.*, 1900.

[2] *Prag. med. Woch.*, 1903, March, No 13., p. 145.

[3] *Gesellsch. f. inn. Med. Wien.*, March 12, 1903.

[4] *Deut. med. Woch.*, 1901, No. 48; and *Centralbl. f. Bakt.*, 1902, No. 1.

and in some persons suffering from entirely different complaints. It has certainly been demonstrated that the test is not so absolutely infallible as was at first expected. Thus Madison[1] finds that there may be marked reaction to tuberculin in cases in which, *post mortem*, no sign of tuberculosis can be found. He also quotes cases of healed tubercle which gave a reaction with the test; while he has met with patients suffering from undoubted tuberculosis who were unaffected by the injections. He places the margin of error at 10 per cent. K. Franz[2] (who considers that there is no danger in the injections) found that the presence of a reaction in healthy persons was very rare, but that in those who are out of health, especially in individuals who are the subjects of syphilis, a reaction to tuberculin is liable to occur. He made experiments on a number of recruits, and considers that on the whole the test is useful and reliable.

At the London Congress of Tuberculosis, E. France related the results which he obtained upon a number of insane patients. Out of 55 persons tested he found that 45 reacted to tuberculin. Twenty-nine of the latter died, and were submitted to necropsy. All of these 29 were proved to be suffering from tuberculosis at the time of death. Among those who did not react to the injections five died, and were examined after death; none of these were found to be tuberculous. These results are very favourable to the use of tuberculin.

Koch himself claims 99 per cent. of correct results from the use of the test. This can hardly be maintained in view of the results of other observers, unless we ascribe special skill to the inventor of the test. Probably the estimate of 10 per cent. of error is not far wrong.

The result of a test with tuberculin may be inconclusive in individual cases in which the question of the tubercular or non-tubercular nature of a particular lesion is at issue. As an instance the following case may be quoted:—The

[1] *American Medicine*, Dec. 20, 1902.
[2] *Wien. med. Woch.*, 1902, Nos. 36–38.

present writers administered 0·01 cc. to a weakly child of five, weighing only 1½ stone, who was suffering from enlarged joints—with a view to determine the nature of this trouble. A reaction ensued, consisting in a rise of temperature (which had previously been normal) to 103° F., with rather troublesome vomiting. The little patient did not seem to feel ill, but complained a good deal of the sickness, as she could not keep her food down, although she felt hungry. No ill effects ensued, beyond a slight degree of redness and induration at the point of injection, which appeared about the third day, and passed off by the fifth or sixth. The temperature fell by lysis, rising on the evenings of the three ensuing days, but each time to a lower figure than on the previous night No signs of redness or swelling were seen in the neighbourhood of the joints, as should have occurred had the lesion been tubercular: yet the febrile reaction had been marked. This may, however, have been due to some small focus of tuberculosis in the lungs or elsewhere.

Tinker[1] states that if the dose be large enough, even healthy persons react He also points out that different specimens of tuberculin vary much in strength, and that a source of error thus arises in comparing results obtained. He lays stress on the advisability of beginning with small doses.

In view of the unpleasantness of the results of the injections (fever, vomiting, &c.) in many patients, as well as the possibility of exciting an exacerbation of the disease —however remote this possibility may be—we should refrain from making use of this means of diagnosis unless there exist special reasons for its employment.

Definite **contra-indications** to the use of diagnostic doses are the existence of fever, nephritis, hæmoptysis and cardiac failure. Epilepsy is also considered by Bandelier and Roepke[2] to constitute a sufficient danger to make the

[1] *Johns Hopkins Hosp. Reports*, vol. xi., 1903, p. 544.

[2] *Lehrb. der spec. Diagnostik. u. Therap. der Tuberk.*, Würzburg, 1908.

procedure unadvisable, as are also diabetes, arteriosclerosis and amyloid change. It should not be used upon very debilitated persons or during menstruation; while in hysterical patients a rise of temperature after an injection is not of the same diagnostic import as in less emotional subjects. Used with these limitations, and with due regard to the margin of error alluded to, there can be no doubt that we have in tuberculin a valuable assistance in the diagnosis of early or obscure cases of tuberculosis.

Mode of using tuberculin for diagnostic purposes.—Tuberculin (T.O.A.) is generally supplied in small glass bottles containing 1 cc. For use it may be diluted with a 25-per-cent. solution of glycerine, if small doses are needed. Thus to administer 0·01 cc. it may be diluted with 9 cc. of glycerine-solution, and one-tenth of a cubic centimetre of the resulting fluid given hypodermically. The position for the injection is immaterial. Slight redness and œdema may occur at the point of injection, but this passes off without any ill effects.

The advice given by Koch for the diagnostic use of tuberculin is as follows: It is necessary to observe the course of the patient's temperature carefully for a day or two—preferably two—before the injection is given, in order to make sure that the daily excursion is within moderate limits. A temperature of 100° F. is a contra-indication to the use of tuberculin, as not only does the existence of such a degree of fever render it difficult to ascertain the exact effect produced by the injection, but the condition of such febrile cases is sometimes depressed by the remedy. If the patient is suitable in the above respect, it is necessary to take into account also his general state of strength or weakness, in order that the dose of tuberculin may be modified accordingly. Delicate individuals receive for a first injection 0·01 of a cubic centimetre, whereas those who appear to be in fair health may at once receive 0·1 cc. The injection is given beneath the skin of the back between the scapulæ. The reaction may be expected in about twelve

hours' time, and Koch prefers to give the injection in the afternoon. If no reaction takes place, a second dose of double the quantity first administered is given on the third day; while if a very slight reaction, such as a rise of half a degree, occurs, the same dose as that which produced this effect is repeated. A much more marked rise of temperature is often seen after this procedure. Koch regards this phenomenon (increased reaction on repetition of a small dose) as very characteristic of tuberculosis. If, however, no effect is produced by the small doses, they may be increased to 0·5 and even to 1·0 cubic centimetre: and this final dose may be administered twice in order to make sure of the absence of a reaction.

Junker[1] advises the following doses for diagnostic purposes:—0·01, 0·05, 0·1, and 0·5 cc. Lowenstein and Kaufmann[2] give 4 doses of 0·02 cc., and if there is no reaction continue with 0·2, 0·5, and 1·0 cc. Roepke[3] finds this repetition of 0·02 cc. insufficient in many cases: he advises successive doses of 0·02, 0·1, and 0·5 cc.

In the case of children, Escherich gives 0·02 to 0·05 cc. to younger, and 0·05 to 0·1 cc. to older patients; Beck gives 0·05 cc. to all under ten years; Leser gives children $\frac{1}{3}$ to $\frac{1}{2}$, and Heubner $\frac{1}{20}$ of the adult dose; Epstein gives children under three years 0·01 cc., and gradually raises the dose by quantities of 0·005 or 0·01 cc.[4]

Trudeau gives the injection as late at night as possible, so as to bring the reaction to a convenient time of day. He insists on the importance of using a fresh tuberculin-solution, which he prepares with $\frac{1}{2}$ per-cent. carbolic-acid solution; it must not be more than three days old. He starts with a dose of 0·1 cc., and if this produces no reaction, goes on to doses of 0·3 cc. and then to 0·5 and 0·7. One cubic centimetre is probably the largest dose which should

[1] *Beitr. z. klin. der Tuberk.*, Bd. vi., Heft 4.
[2] *Zeitschr. f. Tuberk.*, 1907, Bd. x. 17.
[3] *Ibid.*, 1907, x. 5.
[4] Quoted by Schick, *Jahrb. f. Kinderheilk*, 1905, lxi. 811.

ever be administered for diagnosis. M. Beck begins with a dose of 0·1 cc. even in weakly persons. In children under five years of age he starts with 0·03 cc. and goes on to a second dose of 0·1 cc. and then to one of 0·5 cc. In children between five and ten he starts with 0·05 cc., and gives 0·5 as the maximum dose.

Conclusions as to the diagnostic use of tuberculin.—Taking all the evidence at present available, the conclusion appears to be that there is a certain degree of danger in administering a diagnostic injection of tuberculin, but probably not more than in giving an anæsthetic for similar purposes. In no case should we adopt either means if it is possible to make a diagnosis otherwise; but if the matter is one of urgency we should not hesitate to make use of the drug. In doubtful cases of phthisis careful physical examination should be first made, and the sputum should be examined for tubercle-bacilli. If these methods do not clear up the nature of the case, we must consider, from the point of view of the interests of the patient, whether it is necessary to resort to an injection of tuberculin. In the majority of instances probably it is preferable to wait, the patient being meanwhile put into the most favourable possible circumstances to combat the disease, if it be present. Open-air life and plentiful feeding will form suitable treatment for the majority of conditions which are liable to be confused with tuberculosis. But there are a certain number of cases in which the question of the presence or absence of tubercle is of such importance that any means of reaching certainty without further delay should be adopted. Such an instance might be seen in the case of a young man just starting in life, who had to decide on what profession or course of life he should adopt. It might be a question whether it was right for him to enter on an indoor life in a London office, or better that he should emigrate and lead an open-air existence in one of the colonies. Such a question might need an immediate answer, and an injection of tuberculin

might here be not only permissible, but advisable. So, too, might it be in the case of a young woman belonging to a tubercular family, who had perhaps recently suffered from pleurisy, and who sought advice as to the propriety of marrying. But such cases will constitute the minority of those met with in practice. The test should not be used indiscriminately, merely for our own satisfaction. The danger run may be minimal, but for such a purpose we have no right to run any danger at all. It is not justifiable to begin with large doses of the drug.

Special modes of administering tuberculin for diagnosis.—Von Pirquet[1] has suggested the inoculation of tuberculin into the skin instead of hypodermic injection. For this purpose the skin is cleaned as if for a surgical operation; a drop of tuberculin is placed upon it: and through this a series of scratches are made as in vaccination. A "control" lesion is made by scratching with another aseptic needle through a drop of sterile saline solution at another point. In tuberculous subjects this procedure is followed by an inflammatory reaction at the seat of inoculation with the tuberculin: this varies from slight redness round the scratches to an indurated papule and even a vesicle or group of vesicles, which may subsequently undergo desquamation **(cutaneous reaction)**. The reaction usually occurs within twenty four hours: it is occasionally delayed, and may be at its maximum on the third or fourth day. A certain amount of pigmentation may occur, and persist for several weeks. There is no danger in this procedure, but its value as a test for the presence of tuberculosis is disputed. Morro[2] employs inunction of old tuberculin worked up with an equal bulk of lanolin to produce a similar reaction **(percutaneous reaction)**.

Calmette, of Lisle, and Wolff-Eisner, of Berlin, independently suggested the instillation of tuberculin into the conjunctival sac as a test for the presence of tuberculosis.

[1] *Deut. med. Woch.*, May 23, 1907.

[2] *Münch. med. Woch.*, 1908, xlv. 209.

The procedure is generally known as Calmette's **ophthalmic reaction.** It can be performed with a solution of old tuberculin (1–10 per cent., Wolff-Eisner); but owing to the irritant action of the glycerine contained in this preparation, a solution of the precipitate obtained by treating tuberculin with absolute alcohol is generally employed. This can be obtained commercially in a form suitable for making the solutions (p. 257). A drop of the fluid is placed in the hollow formed by drawing down the lower eye-lid, and the patient is directed to hold the head back for a minute or so, in order that the tuberculin may not at once escape. In tuberculous subjects an inflammatory reaction ensues within twelve to twenty-four hours. The untreated eye serves as a control for comparison. As soon as the reaction has been observed, a lotion of boric acid should be prescribed and used until the inflammation has subsided.

A large number of observations have now been made on the use of this reaction clinically. Fortescue-Brickdale[1] collected the statistics relating to over 4,000 cases, with the following results:—Of 1,623 cases clinically tuberculous, 1,419, or 87·4 per cent., gave a positive reaction; of 1,931 clinically non-tuberculous, 214, or 11·1 per cent., were positive; and of 710 which were doubtful, 275, or 38·7 per cent., gave the reaction. From this it would seem that the test is of considerable value as a means of recognising the existence of tuberculosis, but is not infallible. Our personal experience bears out this view.

On the other hand, it must be admitted that the procedure is not entirely free from risk. In an exceedingly small number of cases a severe conjunctivitis may result, and may last for fourteen days or more, causing the patient considerable inconvenience and even suffering. In rare instances mechanical injury to the conjunctiva has appeared to be the starting-point of phlyctenular ulceration,[2] and in one case an incised wound of the cornea caused by the point of

[1] *Bristol Med.-Chir. Journ.*, 1908, xxvi. 112.

[2] *See* Butler, *Brit. Med. Journ.*, 1908, ii. 304.

the pipette during instillation of the tuberculin was followed by sloughing of the cornea, hypopion, and destruction of the globe. Then, too, the solution, when exposed to the air, is an excellent cultivation-medium for bacteria, and the introduction of septic material into the conjunctival sac may give rise to troublesome symptoms. Care must therefore be taken to use sterile solutions. Any existing disease of the eye must be held an absolute bar to the use of the test, as it is impossible to foresee the extent of the inflammation that will ensue. Wolff-Eisner,[1] however, does not regard conjunctivitis as a contra-indication; and Stephenson[2] even recommends the use of the test for cases of suspected tubercular disease of the eye.

On the whole, we are inclined to consider Calmette's test as of great value, as it is free from the risk of causing severe constitutional disturbance and consequent ill effects on the tubercular process elsewhere, and the danger of causing injury to the eye itself is exceedingly small.

THERAPEUTIC USE OF TUBERCULIN

We have already mentioned that it was as a cure for consumption that tuberculin was first announced to the world, and that when the extravagant hopes thus raised were disappointed, the pendulum swung too far in the opposite direction, and the valuable properties of the preparation were overlooked. Some attempts were indeed made to maintain the value of tuberculin in the treatment of lupus; but as a remedy in other kinds of tuberculosis it fell into entire disuse. Yet there is little doubt that in some at least of the varieties of lupus very favourable results may be obtained by a proper use of tuberculin, while in pulmonary tuberculosis good effects are now once more claimed for this remedy. As we have already pointed out, the respective actions of the old tuberculin

[1] "The Ophthalmic and Cutaneous Diagnosis of Tuberculosis," Tr. by Robert, 1908.

[2] *Brit. Med. Journ.*, 1907, ii. 1038.

and of the new (T.R.) are quite different; hence they must be considered separately.

Tuberculin in the treatment of lupus vulgaris.—Very good results were claimed in this disease from the use of the *old tuberculin* when it was first introduced (1890). Koch[1] in his original paper writes as follows: "A few hours after the injection into the skin of the back . . . the lupus-spots begin to swell and redden; and this they generally do before the initial rigor. During the fever swelling and redness increase, and may finally reach a high degree, so that the lupus-tissue becomes brownish and necrotic in places. Where the lupus had been sharply defined we sometimes found a much-swollen and brownish spot surrounded by a whitish edge about a centimetre wide, which again was surrounded by a broad band of bright red. After the subsidence of the fever the swelling of the lupus-tissue decreases gradually, and disappears in about two or three days. The lupus-spots themselves are then covered by a crust of serum, which filters outwards and dries in the air; they change to crusts, which fall off after two or three weeks, and which sometimes leave a clean cicatrix behind after one injection. Generally, however, several injections are required for the complete removal of the lupus-tissue. . . . There is no question of the destruction of the tubercle-bacilli in the tissues; it is only the tissue enclosing the tubercle-bacilli which is affected by the remedy."

Striking results were also recorded by other observers. Thus Saundby, Simon, and Gilbert[2] in a communication to the *Birmingham Medical Review*, though speaking cautiously of the results achieved by the use of the remedy, yet allude to remarkable improvement as taking place in this disease; and Heron, at the Medical Society of London, stated that this was so marked that tuberculin would soon be regarded as an essential in the treatment of lupus.

[1] *Deut. med. Woch.*, 1890; *Brit. Med. Journ.*, 1890, ii. 1193.

[2] *Brit. Med. Journ.*, Epitome, Dec. 20, 1890, p. 92.

Barling[1] recorded fourteen cases of lupus treated by this means, of which four were very much improved, eight considerably so, two slightly benefited. Soon, however, less favourable reports began to come to hand. It was found that, though the first effects were encouraging, relapses were very liable to occur. Radcliffe Crocker pronounced the remedy disappointing on the whole, and this verdict was generally accepted. Consequently tuberculin fell into disuse among the body of the profession as a method of treating lupus.

A few cases are, however, still recorded from time to time in which good results are obtained from the use of the old tuberculin. As an example, we may quote the salient facts of a case reported by E. F Maynard,[2] which illustrates the use of the remedy. The patient was a cook, aged forty, who had suffered from lupus of the nose for some time, and had been treated for the past three years by scraping and cautery (acid nitrate of mercury, fuming nitric acid, &c.) without permanent benefit. The disease was advancing, and had involved the septum nasi. "Old tuberculin" was administered, beginning with doses of 0·001 cc. injected into the arm. The site of injection became red, swollen and painful, and the temperature rose from 99·8° F. to 102·4° F. There ensued headache, nausea and feeling of illness, and the nose became painful, red and swollen. The doses were increased gradually. After 0·005 cc. had been reached there was no further reaction till 0·007 cc. was given Then again no reaction occurred till a dose of 0·03 cc. was reached. After 0·09 cc. had been administered, no further reaction was seen, though 0·1 cc. was given several times over. The disease healed up entirely, and no relapse had occurred seventeen months afterwards.

In the above case the treatment by tuberculin alone, without the adoption of any other measure, seems to have

[1] Quoted in *Brit. Med. Journ.*, leading article, April 25, 1891, p. 922.

[2] *Brit. Med. Journ.*, 1900, ii. 1777.

effected a cure. The majority, however, of those who are in favour of the use of tuberculin recommend that it should be employed along with surgical measures, such as scraping. In some instances it has been combined with the administration of thyroid extract, apparently with good results.

On the introduction of the *new tuberculin* (T.R.) it was tried in lupus by a number of observers. Thus Bussenius [1] reported three out of four cases improved, and Worner [2] four patients all benefited, especially two who suffered from lupus hypertrophicus. Doutrelepont [3] treated fifteen cases, with improvement in all, and van Horn [4] ten with equally good results.

Adrian gives a detailed account of his treatment of twelve cases, of which eight were apparently cured, and four did not entirely yield to the remedy. The following table is taken from one of his articles, the results being added in a separate column :—

Case.	Sex and Age.	Dose.	No. of Injections.	Duration of Treatment.	Result.
1	Female 19	1/1000 increased to 20	50	132 days	Cured.
2	,, 20	1/500 ,, 20	43	129 ,,	,,
3	,, 17	1/500 ,, 20	35	115 ,,	,,
4	,, 39	1/1000 ,, 20	47	143 ,,	,,
5	,, 27	1/500 ,, 20	36	114 ,,	,,
6	Male 32	1/500 ,, 20	36	87 ,,	,,
7	Female 14	1/1000 ,, 20 (× 4)	55	193 ,,	,,
8	,, 6	1/1000 ,, 20	61	216 ,,	,,
9	,, 48	1/500 ,, 12	67	199 ,,	Not cured
10	,, 14	1/500 ,, 2·5	68	156 ,,	,,
11	,, 35	1/500 ,, 2/10	31	84 ,,	,,
12	,, 56	1/1000 ,, 4/10	49	155 ,,	,,

The rise of temperature following the injections was

[1] *Deut. med. Woch.*, 1897, No. 28, p. 441.
[2] *Ibid.*, No. 30, p. 476.
[3] *Ibid.*, No. 37, p. 537.
[4] *Ibid.*, No. 39, p. 625.

generally marked; and sometimes a rigor occurred. The headache and general feeling of illness were parallel to the rise of temperature. The latter was more often met with after use of some particular specimens of tuberculin, and was more marked in some patients than in others. It was found that, if one dose had produced too great a reaction, it was necessary to reduce the amount used to a figure very much lower, even to a point below that at which no reaction had previously been met with. Transitory febrile albuminuria was not uncommon, and in one case severe albuminuria occurred. Hyaline casts were sometimes found in the urine. In no instance did a local abscess ensue at the site of injection, nor was there urticaria, herpes, or enlargement of glands. No local reaction is seen at the site of the lupus-lesions when the new tuberculin is employed—a contrast with the old tuberculin.

In spite of these apparently good results Adrian is not very favourable to the use of tuberculin (T.R.). It is exceedingly expensive, which is undoubtedly a drawback to its use. Adrian used altogether 188 cc. of the fluid in a total of 578 injections, the cost being 1,598 marks, or, approximately, £80. He recommends the employment of surgical measures as well as the tuberculin, and does not consider the new preparation any better than the old.

Mayer[1] practically agrees with this verdict, holding that tuberculin may do good in lupus, but that it is not superior to ordinary measures; while Bussenius and Cossmann[2] report that no constant improvement occurs in all cases. "It cannot be denied," they write, "that Koch's T.R., injected in accordance with Koch's directions, may have a good effect on a focus of lupus; yet our failures and the negative results recorded by others show that such a favourable result is not an absolute certainty." On the

[1] *Arch. f. Derm. u. Syph.*, Bd. xlii., 1898, p. 267.

[2] "Das Tuberculin T.R. und sein Wirkung," Berlin; Hirschwald, 1898. *Cf.* Bussenius, *Deut. med. Woch.*, 1897, No. 28, p. 441.

other hand, Brocchieri [1] considers that the new tuberculin is superior to the old in the treatment of lupus, and may succeed where the latter has failed. He thinks that the spread of the disease is prevented by its employment. The duration of treatment should not be less than one year. More recently favourable reports of its value have been given by Bulloch [2] and by Darier.[3] Bandelier [4] found benefit to result from the use of "perlsucht tuberculin."

On theoretical grounds it would seem reasonable to make use of the two forms of tuberculin in conjunction, using the old preparation until the necrotic tissues are thrown off, and then administering the new tuberculin in order to produce immunity, and thus prevent subsequent relapse and spread of the disease.

Tuberculin in tubercular laryngitis.—Very much the same results were obtained by the use of the *old tuberculin* in laryngeal phthisis as in the case of lupus. Good effects were at first reported, as by Struebing,[5] who recorded a case in which great benefit ensued as the result of this treatment. There was at first a period in which there were increased hoarseness, and pain in the throat—effects of the local reaction. After nine injections the surface of the lesions looked cleaner and healthier, final cicatrisation being produced after 43 doses of the remedy. The ulceration ultimately seemed to be entirely cured. Lennox Brown [6] reported Gerhardt's results in 19 cases, 17 of which were much improved, only two failing to receive any benefit. Senator [7] also reported marked improvement in cases which he had treated. The same doubt, however, as to the permanence of the good effects produced by tuberculin exists in this disease as in lupus, and it is not now often employed.

[1] *Il Policlinico*, 1898, No. 21, p. 489.
[2] *Lancet*, Dec. 2, 1905.
[3] *Ann. de Dermatol.*, 1905, p. 249.
[4] *Beitr. z. Klin. der Tuberk.* Bd. vi. 115.
[5] *Deut. med. Woch.*, Oct 8, 1891.
[6] *Brit. Med. Journ.*, 1890, ii. 1485.
[7] *Berl. klin. Woch.*, Dec. 10, 1890.

Soon after the *new tuberculin* was introduced, Hersfeld[1] recorded seven cases in which he made use of it. He noted that the solutions keep badly, and that a glycerine solution is more painful to the patient than a saline solution. Bandelier and Roepke[2] find the remedy of great value in this affection, practically doing away with the need for local treatment in early cases. Pottenger[3] found von Ruck's tuberculin of use, and Krause[4] used the emulsion of bacilli with advantage.

We have personally treated six cases of tuberculous laryngitis with T.R. and in four of these, where the disease was localised to the larynx, complete recovery, with strong, though harsh, voice, took place; but in two cases associated with extensive lung-mischief, although certain laryngeal improvement at first followed the use of the remedy, death ensued within twelve months.

Tuberculin in disease of bones and joints.—The *old tuberculin* produces phenomena of swelling and redness around tuberculous joints, just as it does around patches of lupus. Koch[5] reported as follows in his original paper:—"Glandular, bone-, and joint-tuberculosis were similarly treated, large doses at intervals being employed. The result was the same as in the lupus-cases—a speedy cure in recent and slight cases, slow improvement in severe cases." In spite of encouraging results recorded by some authors at first, the general verdict was ultimately unfavourable to the use of tuberculin in these cases, most observers apparently agreeing with Edmund Owen that the final results gained were no better than could be produced by rest alone.

Of the *new tuberculin*, Adrian[6] reported that it had no effect on disease of bone or glands. On general grounds it

[1] *Deut. med. Woch.*, 1897, Aug. 19, p. 543.
[2] *Op. supra cit.*
[3] *Am. Journ. Med. Sci.*, Dec., 1906.
[4] *Münch. med. Woch.*, 1905, No. 32.
[5] *Loc. supra cit.*
[6] *Op. cit.*

seems unlikely that, if it raises the general resisting-power of the body, it should have no effect on these special forms of the disease. No doubt it is difficult to ascertain the exact amount of improvement produced by it, as it has no local effect of a visible kind. Theoretically it would seem that the new rather than the old tuberculin should be tried in these cases, as they are deeply-seated, and there is no means of escape for the necrotic material, if it be cast off as a result of treatment with old tuberculin.

Recently good results have been reported by Gray[1] and by Low.[2] A case of hip-disease benefited by tuberculin is recorded by Crofton,[3] and our own observation of a considerable number of cases, treated either in the wards of a hospital or as out-patients, leads us to regard T.R. as a most valuable remedial agent.

Tuberculin in ophthalmic disease. — Eyre and Ormond[4] record complete cure following the use of T.R. in a case of extensive tuberculous disease of the conjunctiva. Reuchlein[5] reports favourably on the use of tuberculin (T.R.) in ocular diseases (iritis, keratitis, disease of the ciliary body). Erdmann[6] also used this method of treatment with advantage, and we have ourselves seen considerable benefit accrue in cases of tubercular iritis, of tubercles in the choroid, and of tuberculous periostitis of the orbit.

Tuberculin in genito-urinary and peritoneal disease.—In the experience of the writers, tuberculin (T.R.) finds its most successful application in tuberculous infections of the genito-urinary tract—indeed, where the disease is bilateral and involves the bladder as well, so that surgical measures are out of the question, the administration of tuberculin holds out the only hope of prolongation of life.

[1] *Lancet*, 1906, i. 1099.
[2] *Brit. Med. Journ.* 1908, i. 550.
[3] *Lancet*, 1908, ii. 731.
[4] *Trans. Ophthalm. Soc.*, 1907, p. 27.
[5] *Klin. Monatsh. f. Augenheilk.*, 1906, i. 352.
[6] *Münch. med. Woch.*, 1907, p. 671.

One of our patients, whose life could to all appearances be measured by weeks only, recovered sufficiently to lead a useful life for another $2\frac{1}{2}$ years.

Pardoe[1] recommends the use of tuberculin in cases of tuberculosis of the bladder, ureters and kidneys. Birnbaum[2] also reports good results in cases of tuberculosis of the bladder, kidney, and uterine adnexa, and in tubercular peritonitis. Bandelier and Roepke[3] confirm the value of tuberculin in the peritoneal affection.

Tuberculin in pulmonary tuberculosis.—In the treatment of tubercular disease of the lung, the original tuberculin has obvious drawbacks. It causes reaction of the tissues round the lesions, and consequent casting-off of diseased material. In a deeply-situated organ such as the lung, the cast-off matter cannot be readily expelled, and danger may ensue. Hence in pulmonary disease it would seem preferable to employ the *new tuberculin*: but both varieties have been used, and the writers do not always distinguish between them. Hence they must be considered together.

In a communication to the South California Medical Society, Pottenger[4] gives the result of his own experience, along with much information gained in answer to questions addressed to some of the principal authorities on the treatment of tuberculosis as to their experiences with this remedy. He finds that of those who have actually used tuberculin 60 per cent. are in favour of it as a means of treatment. Those who recommended the procedure based their advice on as many as 5,742 cases treated, whereas those who were of the opposite view had only a material of 813 cases to rely upon; indeed, only four of those who denied the value of the drug had at all an extensive experience of

[1] *Lancet*, 1905, ii. 1766.

[2] *Centralbl. f. Gynäk.*, 1907, No. 3.

[3] *Op. supra cit.*

[4] *Therapeutic Gazette*, 1903, p. 163. The references to other authors in the following paragraphs are taken from this article.

its use. This is, of course, only what might be expected, as those who found that they were getting no good results would cease using the tuberculin, while those who found it of value would persevere. It is noteworthy that of the four who had tried it extensively, and yet reported unfavourably on the whole, not one was actually opposed to its use, and all had apparently seen some cases at least in which good had been done.

In the majority of instances, those who had abandoned tuberculin-treatment had not at any time given it an extended trial; whereas Petrushky maintained at the Berlin Congress that, in order to produce lasting effects, the treatment should extend over several years, a course of a few months being taken each year.

Coming to actual results claimed for treatment with tuberculin in addition to ordinary measures, we find that, in addition to his own success, Pottenger quotes five other physicians who claim to have cured 100 per cent. of those cases which came under treatment with tuberculin in the earliest stage of the disease (Jessen, Turban, Wilkinson, Klebs, Petrushky). Von Ruck claims 93 per cent., Trudeau 83 per cent., and Rembold 75 per cent. of cures in similar cases. In all, 589 cases treated with tuberculin come under consideration, with a proportion of cures equivalent to 84·2 per cent. On the other hand, among 611 collected cases which were treated in the ordinary way without the aid of "culture-products," 391, or 64 per cent., were regarded as cured.

The results obtained by individuals with and without tuberculin, as quoted by the same writer, are of considerable interest. Trudeau, in his first report on the remedy, recorded 24 cases treated with tuberculin, with a percentage of cures of 83. Among 113 cases treated without it he cured 72 per cent., giving a difference of 11 per cent. in favour of the remedy. More recently he gives the results of 94 cases, 47 treated with, and the same number without, tuberculin; of the former group 41 were cured, of the

latter 36—again a small difference in favour of Koch's preparation.

Turban gives details of his results in cases which came under treatment in the first, second, and third stage[1] of the disease respectively. Taking the last first: he found that, whereas the mortality in cases treated without tuberculin was 50 per cent. within a period of two years, among those treated with tuberculin only 25 per cent. died within the same time-limit. He did not find that tuberculin had any tendency to induce attacks of hæmorrhage in these cases, nor did it ever give rise to a generalised tuberculosis. Tubercle-bacilli disappeared from the sputum in four cases out of 21 in which tuberculin was used. Of course a real cure was not to be hoped for in patients coming for treatment at so advanced a stage of the disease.

Of 48 patients in the second stage of the disease, treated with tuberculin, 36 were alive four years afterwards, whereas of 152 who did not receive injections 107 survived for the same length of time. The figures do not themselves prove much in favour of tuberculin, but Turban considers that the actual condition of the various patients afforded strong evidence of its value. Of cases which came under treatment in the first stage of the malady, Turban, as

[1] For purposes of classification in statistics of sanatoria, &c., pulmonary tuberculosis is divided into three stages. Different authorities have devised slightly different methods of classification. That of Turban, which may be taken as typical, is into—*First stage*: Cases in which only one lobe is affected, or only portions of two lobes equivalent to one lobe in extent. *Second stage*: Cases in which two lobes are extensively involved. *Third stage*: Cases in which the disease is still further advanced. It will be seen that this classification is purely arbitrary, and merely affords a rough indication of the severity of individual cases. It does not correspond at all with the well-known pathological division into (1) Stage of tubercular deposit; (2) Consolidation; (3) Excavation or cavity-formation. It is practically impossible to ascertain with any exactitude the extent of the pathological changes in the lung from a study of the physical signs; the pathological classification is therefore not available for practical use.

already stated, claims 100 per cent. of cures. In all the cases in this stage of the malady in which tubercle-bacilli were at first found in the sputum, they disappeared under treatment. Taking this last as a test of the value of tuberculin, he shows that of a total of 86 cases so treated, 45 (52 per cent.) were permanently freed from the organisms, whereas of 241 patients not so treated, 95 (39 per cent.) only were similarly benefited.

Denys has made trial of tuberculin alone, without the aid of other remedial measures, such as rest, open air, and medicines. He claims to have cured in this way 29 per cent. of his cases (174), and greatly benefited another 42 per cent. As was previously pointed out, there is no reason, except for purely experimental purposes, to suspend ordinary hygienic measures during the administration of tuberculin; in order to produce the maximum of advantage to the patient the two should be combined.

Wurtzen[1] records good results obtained in ten cases with the *old tuberculin*, given according to the rules advised by Goetsch,[2] viz. never to inject febrile patients; never to increase the dose till the previous amount can be tolerated without reaction; and to insist on rest in bed on the day of treatment and the following day.

There is no doubt of the fact that immunity to tubercular infection can be produced in animals by injections of tuberculin. Of its action on human beings it is possible to obtain some distinct proof by the increased power of agglutinating the *Bacillus tuberculosis*, seen in the blood-serum of such patients (*see* p. 289), and by its effects on the opsonin-index. It is not, indeed, certain that the immunity of the individual is directly proportionate to either of these properties, but there is evidence to show that they constitute some measure of the resistance. It seems, however, that as a result of injections of tuberculin

[1] *Tuberculosis Bull. Mens.*, Feb. 1904, p. 53.

[2] *Deut. med. Woch.*, June 20, 1901.

an individual may acquire a tolerance of this drug without an accompanying immunity to tuberculosis.

Within the last few years, largely owing to the work of Wright, a considerable impetus has been given to the use of tuberculin in pulmonary tuberculosis, and with the adoption of smaller doses the proportion of successful results would seem to have risen. There seems, indeed, to be a consensus of opinion that in suitable cases the value of the remedy is beyond question. There is, however, a difference of opinion as to what cases are best adapted for the treatment. Thus Amrein[1] and Roemisch[2] would restrict the use of the remedy to chronic cases without fever, and Lawson and Stewart[3] agree with this advice; whereas Krause[4] and Hammer[5] do not regard fever as a contra-indication. Hæmoptysis is not always looked upon as a bar to the cautious use of the new tuberculin for remedial purposes, though it is so to the diagnostic use of the old tuberculin: it is even suggested that tuberculin is valuable as an agent for arresting hæmorrhage. A few writers still recommend the old tuberculin for purposes of treatment (Foss,[6] Jacquerod[7]), but "T.R." is the form usually adopted. The emulsion of the bacilli is preferred by some (Krause,[8] Elsaesser,[9] Poppelmann[10]), and has also been used by Wright.

Our own experience is in favour of restricting the use of tuberculin, as a rule, to cases of quiescent disease, with little or no fever. Great caution must be observed and very minute initial doses given, if it be decided to

[1] *Beit. z. Klin. d. Tuberk.*, Bd. iv., Hft. 2.
[2] *Münch. med. Woch.*, 1906, No. 3.
[3] *Lancet*, 1905, ii. 1679.
[4] *Münch. med. Woch.*, 1905, No. 32.
[5] *Ibid.*, 1906, p. 2423.
[6] *Zeitsch. f. Tuberk.*, Bd. vi., Hft. 5.
[7] *Rev. Méd. de la Suisse Romande*, 1906, No. 2.
[8] *Op. cit.*
[9] *Deut. med. Woch.*, 1905, No. 48.
[10] *Berl. klin. Woch.*, 905, No. 36.

administer the remedy to patients who suffer from pyrexia, as considerable harm may be done by doses which excite any strong reaction. The question of dosage is discussed below.

Method of administration of tuberculin for therapeutic purposes.—The new tuberculin is supplied in liquid condition. It is an opalescent liquid similar in appearance to the mixture of five or six drops of milk with half an ounce of water. It must be kept in a cool, dark, and dry store. The solution contains the soluble protoplasm from 10 mg. of dried bacilli in each cubic centimetre. The actual amount of solid substance taken into solution amounts to 2 mg. Koch advised, for the treatment of phthisis with the old tuberculin, that the initial dose should be 0·001 cc. and that this should be repeated till no reaction followed its use. Then the dose should be raised to 0·002 cc. and so on, rising to 0·01 cc. &c. In strong individuals it might be possible to raise the doses more quickly. In lupus the first doses might be larger, 0·01 cc., gradually raised.

With the new tuberculin (T.R.) the initial dose, according to Koch, should be 0·002 mg. in fairly strong persons (equivalent to 0·0002 cc. of the preparation, and containing actually 0·0004 mg. of solid substance); in very weakly subjects it is well to start with 0·001 mg. The dose is to be repeated every other day, increasing at such a rate as to avoid reaction as far as possible; the amount may usually be doubled each time.[1]

[1] The directions issued by the makers with the bottles of tuberculin may be summarised thus :—

The treatment is generally commenced with a quantity equivalent to 0·0002 cc. of the preparation supplied. If a reaction appears, the dose must be reduced.

The injections are made subcutaneously about every second day, the dose being raised so gradually that a rise in temperature of more than half a degree is as far as possible avoided. Any febrile symptom caused by the injection must have entirely disappeared before a fresh injection is made. With doses of 5 milligrammes of solid substance and

Doutrelepont,[1] who tried this method, came to the conclusion that this rate of increase was too rapid. He advised that 0·002 mg. should be used for the first dose, 0·004 for the second, 0·006 for the third, and so on, up to 0·02 mg. Then the dose is to be increased by 0·02 each time up to 1 mg. The dose should not be repeated till the temperature has fallen to normal after the previous reaction, and the largest dose used by Doutrelepont was 4 mg. (0·4 cc. of the preparation). The fresher the solution, the more likely is the occurrence of a marked reaction.

Rosenberger[2] gives 0·002 mg. of the new tuberculin to start with. The administration is followed by little febrile disturbance, but there may be some headache and sleeplessness. The appetite is increased by the treatment. The dose is gradually raised till 1 mg. is reached; then the old tuberculin is begun, the initial dose of the latter being 0·01 cc.

Recent practice in this country has tended in the direc-

upwards it is not advisable to make more than two injections within the week, and with still larger doses not more than one. The individuality of the patient has generally to be taken into account.

For injection, those parts of the body should be selected at which large folds of skin may be raised.

For dilution of the liquid, 20 per cent. glycerine solution should be employed. This is prepared by boiling 20 cc. pure glycerine with 80 cc. distilled water for 15 minutes, and then cooling thoroughly before use. The dilutions are preferably made in the following manner:

1. With a 1 cc. pipette, calibrated to $\frac{1}{10}$th, 0·3 cc. is withdrawn from the bottle, and mixed with 2·7 cc. 20 per cent. glycerine solution, making a 10 per cent. dilution.
2. From this 10 per cent. dilution 0·1 cc. is taken and made up to 10 cc. with glycerine solution. Thus a 1 per mille dilution of the original fluid is obtained. Two divisions or $\frac{2}{10}$th cc. of a Koch or Pravaz syringe of this dilution therefore contains the initial dose, 0·0002 cc. of the original fluid.

Dilutions which present a turbid appearance, or show a deposit which does not dissolve upon shaking, must not be employed. Generally the dilutions keep well for a fortnight in a cool and dark place.

[1] *Deut. med. Woch.*, Aug. 19, 1897, p. 537.

[2] *Centralbl. f. inn. Med.*, 1903, No. 19, p. 465.

tion of giving much smaller doses of tuberculin than were formerly used. Thus, whereas 0·002 mg. of T.R. was considered a small amount to give as an intitial dose, now 0·001 mg. would be looked upon as a large dose, and 0·00025, 0·0002 or 0·0001 mg. are more often given at first. Still more minute quantities are believed by some physicians to be capable of producing a definite reaction and to form effective doses for therapeutic use (*e.g.* 0·00005, 0·00002, or 0·00001 mg.). The object to be aimed at is to administer that amount which will just fail to produce an appreciable reaction in the form of a rise of temperature, headache, sleeplessness, or other constitutional disturbance. The first doses therefore should always be small (*e.g.* 0 00005 mg.), and they should be raised very gradually until some signs of reaction occur. Thus, a course of injections might be given as follows: 0·00005, 0·0001, 0·0002, 0·0004, 0·0005, 0·001 mg. Or a small dose such as 0·0001 mg. may be maintained throughout. We have seen a harmful reaction ensue after a dose of 0·001 mg.[1]

Another change in the method of administering tuberculin which has been effected in recent years is the tendency to prolong the intervals between the separate doses. Thus, whereas they were originally given daily, or on alternate days, it is now usual to let an interval of 7, 10, 14, or even 21 days elapse between the injections. Sometimes it is preferred to give very small doses on two days running, and then to wait for an interval. No definite rules can be laid down for all cases: each must be judged according to the special phenomena presented.

That tuberculin given *by the mouth* is absorbed and produces similar effects to those which follow hypodermic injection was affirmed by Freymuth[2]; but Köhler,[3] who tried

[1] These doses represent the weight of tubercle-bacilli used in their preparation. To ascertain the actual weight of soluble protoplasm actually contained in each, it is necessary to divide the figure given by 5.

[2] Quoted by Köhler.

[3] *Zeitschr. f. Tuberk.*, 1907, Hft. 4.

this method of administration for therapeutic purposes, was not impressed by its advantages. Calmette and Breton[1] state that it is also absorbed by the bowel. Recently Latham[2] has used the oral method extensively, and upholds the value of this procedure. For such use the tuberculin (T.R.) is diluted with normal saline solution. It can be given in milk, or in any flavoured water if this is preferred, the same doses being employed as are used for hypodermic injection. On the whole, we are not inclined to recommend this method of administration, and believe that the evidence adduced in its favour is fallacious.

A curious feature that is sometimes observed in the course of treating tubercular lesions with tuberculin is that while the original lesion is undergoing marked improvement, apparently as a result of the remedy, some new focus of tuberculosis may arise elsewhere, and may even run a rapidly progressive course. It is difficult to explain this occurrence in view of the general increase in resistance that should follow the appropriate use of tuberculin.

Kapralik and Schrötter[3] find that tuberculin is also absorbed readily when administered by inhalation in the form of a spray; but there seems to be no advantage in this inconvenient mode of procedure.

General considerations on the therapeutic effects of tuberculin.—There can be little doubt that the want of success which was met with by the majority of those who used tuberculin, when it was first introduced to the medical world, was due to ignorance of the nature of the remedy, and of the effects which could be rightly expected of it. Tuberculin has not—and was never supposed by its inventor to have—any power of replacing tissue already destroyed by the disease; nor can it do anything to check the action

[1] *Compt. Rend. Acad. Sci.*, cxlii. 11. *Cf.* Lissauer, *Deut. med. Woch.*, 1908, p. 1335.

[2] *Proc. R. Soc. Med.*, 1908, *Clinical Sect.*, p. 100.

[3] *Wien. klin. Woch.*, 1904, No. 12, p. 583.

of other bacteria, such as streptococci or staphylococci, which may have secondarily invaded the cavities in the lungs or other ulcerated lesions. We thus see that, just as in serum-treatment it is important to administer the dose of antitoxine before the poisons of the bacteria have gained too long a start and entered into combination with the cells, so it is equally necessary to make use of tuberculin (if at all) in the early stages of tubercular disease, before the substance of the affected organ has been so extensively destroyed as permanently to cripple the infected individual by the loss of an important structure. It is therefore in incipient tuberculosis that we must look for the most marked results from the administration of tuberculin. In more advanced cases it may, indeed, be of assistance in increasing the resistance of the body to the tubercle-bacilli, but permanent lesions will necessarily remain, and no cure in the truest sense can be hoped for.

A further reason for the original failure of tuberculin to come up to the expectations formed of it, was that reliance was placed upon it, alone and unaided, to accomplish the cure of tubercular disease. Its value is now recognised as an adjuvant to other remedial measures, not as a specific curative agent, such as, for example, mercury is for syphilis, or quinine for malaria.

Great advances have been made in our knowledge of the nature of tuberculosis, especially in the recognition of the value of a plentiful supply of nourishment, and of fresh, unbreathed air for the lungs. These hygienic measures are not to be neglected during the employment of tuberculin. Used rationally in the light of modern experience, tuberculin is now proving itself a valuable remedy.

AGGLUTINATION OF B. TUBERCULOSIS

Agglutination-test for tuberculosis.—It is found that the blood of a patient suffering from tuberculosis has the power of causing a clumping of tubercle-bacilli,

in the same way as that of a typhoid patient agglutinates the *B. typhosus*. Use was made of this property by Arloing and Courmont[1] as a means of recognising the presence of tuberculosis.

Preparation of the emulsion.—In the case of the tubercle-bacillus, a preliminary difficulty arises, which is not found in working with the *B. typhosus*, in that the former organism, when grown in the laboratory on ordinary culture-media, occurs in masses which are already closely agglutinated. It is necessary, therefore, for the purpose of the test, to prepare the bacilli in some special way so that they are separated one from another. This was first accomplished by Arloing[2] by the following method of procedure: Suitable potatoes are taken and boiled, and slices of them are put into the usual laboratory potato-tubes. At the bottom of the tubes is placed a small quantity of a 6-per-cent. solution of glycerine in water, so that the fluid just touches the lower part of the potato. The tubes thus prepared are sterilised for forty-five minutes in the autoclave. The surfaces of the slices of potato are then inoculated with tubercle-bacilli derived from a human source, and the cultures are incubated at a temperature of 38° to 39° C. On every second day the tubes are tipped up, so that by the inclination of the tubes the glycerine-solution is caused to flow over the cultures on the surface of the potato. Growth occurs rapidly in these circumstances, the resulting masses of organisms being different from the ordinary cultures on glycerine-agar, in that they are soft in consistency, and easily broken up by the application of a glass rod, or by rubbing in a mortar. From these cultures subcultures are made in glycerinated veal-broth (1 per cent. peptone and 6 per cent. glycerine). These are submitted to daily shaking to keep the organisms separate one from another. Even in these cultures it is impossible to prevent a certain amount of clump-formation, but the majority of

[1] *Gaz. des Hôp.*, 1900, p. 1467.

[2] *Comptes Rend. Acad. Sci.*, 1898, cxxvi. 1319.

the organisms present are separate, and the fluid is turbid and fairly homogeneous, with but little sediment.

It is interesting to note that, grown under the conditions indicated, the bacilli are described as motile, some writers even attributing to them a degree of motility equal to that of the *Bacillus typhosus.* Branching forms are also met with. Koch at one time doubted the identity of the bacilli described by Arloing and Courmont with his own bacilli.

Some writers advise that, before they are used for the agglutination-test, the organisms should be transferred through a series of broth-cultures, holding that they thus become more motile, are better separated, and grow more rapidly. Loeb[1] does not recommend this procedure, as he has found that it is impossible to grow the bacilli beyond the fourth generation. This observer has failed to discover active movement in the bacilli, though the usual Brownian movement may be seen.

The broth-culture should be grown for a period of from nine to fifteen days, and then used for the test. Before the eighth day there are not enough bacilli present, while after the fifteenth, spontaneous agglutination may take place, and the reaction with serum is often lessened at this time. The test-fluid can be preserved in a condition fit for use by keeping it on ice, or by the addition of a minute quantity of some antiseptic, *e.g.* formalin (1 : 400), or carbolic acid: both methods may be combined. Some such mode of preservation is necessary, as the labour of making a separate culture for every experiment would be enormous.

Mode of performing the test.—The mode of applying the test is as follows: Clear blood-serum or inflammatory fluid from a patient suspected of tuberculosis is added in varying proportions to a series of tubes of the suspended bacilli. The tubes are placed in an incubator for a period of 2 to 6 hours, inclined at an angle of 45 degrees. If they are allowed to remain for a longer period, for

[1] *Journ. of the American Med. Assoc.*, May 23, 1903, pp. 1, 423, &c.

instance 24 hours, as was at first recommended, normal serum may give rise to a certain amount of agglutination. If the reaction be positive, the serum gradually appears less opaque, a flocculent precipitate falling to the bottom. This is visible with the unaided eye, on examination in a bright light against a dark background. A control experiment should be made, for purpose of comparison, with normal serum. Microscopically, it will be seen that the bacilli are clumped as in the "Widal test" for enteric fever. The test in tuberculosis is not, however, so well marked as in the former disease. Errors may occur owing to the presence of small fibrinous coagula, especially when inflammatory exudates are employed for the test. It is necessary to make certain by means of the microscope that any apparent clumps are in reality formed of bacilli. Staining reagents may be needed to decide in cases of doubt. It must be borne in mind that a certain proportion of clumped bacilli may occur in the cultures, however carefully they are prepared; hence arises the importance of invariably making use of a control experiment.

Simplification of the procedure. -The process adopted by Arloing and Courmont is very long and tedious, and modifications have been suggested for the purpose of simplifying it. Thus, Romberg prepared a suspension of the bacilli by macerating dried tubercle-bacilli with a 1·5-per-cent. solution of caustic soda, and then neutralising with acetic acid. Koch[1] has recently described a still simpler method. He takes an ordinary culture and dries it by pressure between pieces of blotting-paper. A known quantity is then weighed out and rubbed up in a mortar with a weak solution of caustic soda. Instead of this a culture may be dried and triturated in a mortar to a fine powder. A weighed quantity of the powder (0·1 gr.) is macerated with saline solution, added in quantities of a few drops at a time, till the solid culture is diluted to 1 : 100. The solid residue is then separated by the centrifuge, and

[1] *Deut. med. Woch.*, Nov. 28, 1901.

the supernatant fluid is decanted and diluted with a fresh amount of salt-solution, to which a small amount of carbolic acid is added, till the dilution reaches 1 : 1,000. This fluid can be kept without alteration owing to the presence of the carbolic acid. For use it is again diluted to 1 : 10,000, but this last dilution seems unnecessary and almost disadvantageous.[1] If a strongly-clumping serum is added to the fluid in the proportion of 1 : 10 or 1 : 25, agglutination rapidly occurs. This is aided by a moderate degree of warmth, as by holding the test-tube in the hand. The reaction takes place much more quickly in the stronger fluid (1 : 1,000) than in the extreme dilution. The time-limit recommended by Koch is 15 to 20 hours. A good plan is to put the tubes in the incubator over night, and to examine them in the morning. Koch employs dilutions of serum of 1 : 10, 1 : 25, 1 : 75, 1 : 100, and so on, in a series of tubes. The serum is first poured into the test-tube and the fluid containing the bacilli is added, and the mixture shaken up. A control test is always necessary.

Koch advises removal of the serum needed by means of a cupping-glass, while Arloing and Courmont draw blood from a vein and remove the corpuscles by the centrifuge.

Agglutinative power in human beings.—In human beings it is found that the serum of those who are not suffering from tuberculosis may at times possess an agglutinative power. Infants and young children do not seem to give a reaction, but adults may do so. Thus of 30 non-tuberculous persons, five gave a reaction in dilutions of 1 : 25, one at 1 : 50. In one case a subsequent *post-mortem* examination proved the absence of tubercular infection. Of 78 phthisical cases, only 14 gave a positive reaction in dilutions of 1 : 10, one case at 1 : 50, four at 1 : 25. In several cases of tuberculosis affecting other regions (bladder, bone, &c.) no reaction was obtained.

Arloing and Courmont give the following statistics of

[1] Powdered bacilli ready prepared for the agglutination-test may be obtained from Messrs. Meister, Lucius and Brüning.

results: Of 191 persons presenting clinical signs of tuberculosis, 168 or 87·9 per cent. reacted positively, while 23 or 12·1 per cent. were negative. Of 130 cases clinically non-tuberculous, 45 reacted (34·6 per cent), 85 (65·4 per cent.) were negative. Among 41 healthy persons 11 reacted (26·8 per cent.), while 30 were negative (73·2 per cent.). In all these cases blood-serum was employed for the test. Serous effusions gave the following results:—

	Positive.	Negative.
Tubercular pleural effusions (31) ...	23 ...	8
Pleurisy of doubtful origin (16) ...	13 ...	3
Non-tubercular hydrothorax (11) ...	0 ...	11
Tubercular ascites ... ... (13) ...	11 ...	2
Non-tubercular ascites ... (20) ...	0 ...	20

In cases of tubercular meningitis the result was always negative in children, but two adults gave positive reactions. The above figures would suggest that the test may be a valuable aid in the diagnosis of tubercular peritonitis from conditions which produce similar symptoms, such as cirrhosis of the liver and chronic simple peritonitis, if the latter condition really exist. The failure of the reaction in 12 per cent. of clinically tuberculous cases suggests that for ordinary use the test is of doubtful value. The non-appearance of the reaction in cases of tubercular meningitis in children is particularly unfortunate, as this disease is a very insidious one, for which a sure test would be of the greatest service, while it would certainly not be legitimate to make use of tuberculin in such a malady.

The results obtained by Beck and Rabinovitch[1] were very much less favourable for the value of the test. Thus in cattle, among 19 healthy beasts, 12 gave a positive reaction, and among 4 suffering from diseases other than tuberculosis 3 reacted. Among 17 beasts with early tubercle, 6 were negative, and among 22 moderately advanced cases 2 were negative and 6 only reacted in a dilution of 1 : 5, at which point the serum of even healthy cattle may

[1] Quoted by Loeb, *loc. cit.*

cause agglutination. Among 16 very advanced cases 1 was negative and 4 reacted only at 1 : 5. In human beings these observers record that among 17 cases of incipient tuberculosis, only 6 gave a positive reaction, and among 16 advanced cases only 4 reacted. Among 5 doubtful cases which gave a positive reaction with tuberculin, only 1 reacted positively with the agglutination-test. On the other hand, of 31 non-tuberculous cases, 10 reacted positively.

Humbert [1] found the reaction positive in 4 cases of miliary tuberculosis, and thinks the test useful; as do also Sabareanu and Salomon,[2] Vasilescu [3] (diagnosis from enteric fever, influenza), and Grysez and Job.[4] On the other hand Friedmann [5] and Kington and Twichell [6] find it of no value. Simoni [7] found it of use in the diagnosis of tubercular ear-disease, and Pellegrini,[8] who admits that it is uncertain, in surgical tuberculosis.

On the whole, it seems necessary to conclude, on the evidence at present available, that the agglutination-test is of little or no practical use in the diagnosis of tuberculosis. This is the opinion of Koch and of Beck and Rabinovitch. The margin of error is too great for the test to afford trustworthy indications for clinical use. The most hopeful field for further experiments with this reaction is in the diagnosis of tubercular ascitic effusions, in which the fluid is easily obtained, and in which, so far, the recorded results are encouraging.

VACCINATION AGAINST TUBERCULOSIS

Attenuation of tubercle-bacilli.—For the purpose of vaccination against any disease, the first requisite is the

[1] *Rev. de la Tuberc.*, 1904, p. 233.
[2] *Rev. de Méd.*, 1905, No. 7.
[3] *Inaug. Diss. Bucharest*, 1905.
[4] *Rev. de Méd.*, 1906, p. 705.
[5] Abstr. *Centralbl. f. inn. Med.*, 1906, p. 757.
[6] *Amer. Journ. Med. Sci.*, Oct., 1906.
[7] *Gaz. degli Osped.*, May 1, 1904.
[8] *La Clin. Mod.*, 1904. x., Nos. 27, 28.

preparation of an attenuated form of the causal organism, and for a long time it seemed as if it were impossible to reduce the virulence of the tubercle-bacillus. Many observers [1] have, however, now succeeded in the endeavour to attenuate this organism, and in the case of the lower animals it has been claimed that immunity can be produced by means of such cultures. In 1889 Darenburg [2] inoculated rabbits with dead cultures of tubercle-bacilli, and found that, though they were at the time made ill by the injections, yet afterwards they were more resistant to infection with virulent bacilli. In the same year Grancher and Martin [3] prepared a series of cultures of different degrees of virulence, and stated that they had succeeded in immunising rabbits by this means against the disease.

In 1890, Trudeau [4] gave an account of two cultures of tubercle-bacilli of very different virulence. The first was from the lung of a man who had died of miliary tuberculosis. It grew very slowly on glycerine-agar in isolated scaly masses. The second was from a guinea-pig which had been inoculated with bacilli from an old phthisical cavity, and the bacilli had been grown for a long time on artificial media. This culture grew rapidly, forming a thick, creamy pellicle on the surface of the medium. It was much less virulent for rabbits than the former. Trudeau failed, however, to produce immunity by injection either of culture-products or of attenuated organisms. In the year 1894 he announced that rabbits inoculated with avian bacilli seemed to gain a certain amount of additional resistance to the human form. Guinea-pigs, which are scarcely susceptible

[1] Salmon (*Philadelphia Med. Journ.*, June 13, 1903, p. 966) gives an historical summary of the results obtained in attenuating the tubercle-bacillus. The following account is principally taken from his paper.

[2] *Bull. de l' Acad. de Méd.*, Oct. 29, 1889, p. 391.

[3] *Ibid.*, Aug. 20, 1890.

[4] *Trans. Assoc. Amer. Physicians*, 1890, vol. v., p. 183. *Ibid.*, 1894, vol. ix., p. 168.

to the avian bacillus, are not protected by injections of it against infection with other forms.

In 1894 de Schweinitz, by repeated subcultures, had produced a bacillus which was so attenuated that it no longer produced tuberculosis even in guinea-pigs, and by inoculating the animals with these attenuated bacilli, and afterwards with others of gradually-ascending degrees of virulence, he immunised them against bovine bacilli. In 1897 the same observer showed that by injection with human tubercle-bacilli cows could be rendered immune to the bovine bacillus.

It is interesting to notice that the better a variety of the tubercle-bacillus grows on artificial media, the less virulent it appears to be. The artificial pabulum constitutes a new environment to which the organism has to get accustomed, and as it does so it loses its original power of acting as a parasite. This forms a good example of the variation of bacteria according to their surroundings.

Behring [1] claims that by injection of human tubercle-bacilli into cattle he has produced immunity to the bovine form of the disease. The procedure, which he speaks of as "Jennerisation," is harmless to the animals, and they subsequently resist, not only artificial inoculation with their own form of tuberculosis, but also infection in the ordinary course of nature, when they are brought into contact with other animals suffering from the disease. The duration of the immunity thus conferred is not yet certainly known; a second vaccination may be necessary subsequently. Behring suggests that this latter might be performed with modified bovine bacilli.

Friedmann [2] has made use of bacilli derived from the tortoise for immunising warm-blooded animals; and Moeller [3] has experimented with similar bacilli from the slow-worm. Both authors record good results; but the method has not been tried on man.

[1] *Zeitschr. f. Thiermedicin*, Bd. vi., Hft. 5 and 6.

[2] *Therap. Monatsh.*, March, 1904, p. 123.

[3] *Zeitschr. f. Tuberk. u. Heilst.*, Jan., 1904.

In human beings prophylactic injection of attenuated bacilli does not seem to have as yet been attempted, but it may be pointed out that the injection of Koch's new tuberculin (T.R.), and still more of his emulsion of bacilli, is very closely allied to such a mode of treatment, since the remedy consists of the actual bodies of the bacilli, broken up and suspended or dissolved in an indifferent fluid.

It is evident that the danger of using living tubercle-bacilli as a vaccine for human beings is too great to be faced. Maragliano,[1] however, announces that he has prepared a vaccine of a non-living nature, which he has employed on human beings, but the exact mode of preparing this material is not stated in his communication. The use of it is said to result in an increase of the agglutinative power of the blood-serum, and also in a marked leucocytosis. These are the changes that have been observed in the blood of animals which have been immunised against the tubercle-bacillus experimentally. The injections of Maragliano's vaccine are followed in human beings by the development of a small tubercular ulcer at the point of inoculation, accompanied by a form of suppuration which is bacteriologically sterile. There is fever for a few days, but no other ill effect. Behring[2] suggests that it may be possible to immunise young children prophylactically with antibodies derived from animals which have been injected with attenuated bacilli, and also administers tulase (p. 258) in milk for the same purpose.

SERUM-THERAPEUTICS OF TUBERCULOSIS

Various attempts have been made to treat tuberculosis by an antitoxic serum on the lines of that used for diphtheria. To prepare the serum, horses or other animals are injected with gradually-increasing doses of tuberculin or similar toxic products of the growth of the organism,

[1] Communication to the International Medical Congress, Madrid, 1903. *Medical News*, July 4, 1903, p. 1.

[2] *Berlin. klin. Woch.*, March 16, 1903.

Marchiafava's serum.—For the preparation of his serum Marchiafava uses two separate horses: (1) a carrier of the tertian organism by means of a subcutaneous injection of 10 c.c. of blood containing a considerable number of parasites, and (2) a carrier of the quotidian organism by means of a subcutaneous injection of 10 c.c. of blood containing a considerable number of parasites. The serum of the former is used in cases of tertian infection, that of the latter in cases of quotidian infection. The serum is used in doses of 1 c.c. for every kilogram of body-weight, and is usually injected subcutaneously. The immunity produced by the serum lasts for about a week. The serum is said to be harmless, and to have no ill effects on the patient. It is also said to be of value as a prophylactic against malaria.

Marino Zuco's serum.—Marino Zuco prepares his serum from the blood of rabbits which have been inoculated with malarial blood. The blood is taken from the rabbit when the temperature is highest, and is injected into the patient in doses of 10 c.c. The serum is said to be harmless, and to have no ill effects on the patient. It is also said to be of value as a prophylactic against malaria.

Marino Zuco's serum.—Marino Zuco prepares his serum from the blood of rabbits which have been inoculated with malarial blood. The blood is taken from the rabbit when the temperature is highest, and is injected into the patient in doses of 10 c.c. The serum is said to be harmless, and to have no ill effects on the patient. It is also said to be of value as a prophylactic against malaria.

[1] Quoted from a private communication, 1913, p. 5.

[2] Marino Zuco, Rivista di Clinica Medica, 1910, p. 857.

come under consideration, and the uncomplicated cases may be thus tabulated :—

	Total.	Cured.	Improved.	Stationary.	No Effect.
Circumscribed apyretic cases ...	250[1]	95	110	30	35
Circumscribed febrile cases ..	938	168	511	163	96
Diffuse tubercular bronchitis ..	665[2]	91	301	166	106
Advanced phthisis with cavities	712[3]	39	281	102	240

Such results are certainly noteworthy. Mircoli states that the improvement obtained is generally permanent, relapses being infrequent—as if the organism when it had once been assisted to defend itself against the tubercular parasite, were able to continue the struggle successfully for the future. The amount of complement present in the blood is said to be increased, and the antitoxic power is distinctly raised. This is not a mere passive immunity, due to the actual doses of antitoxine administered, as the increase is much greater than can be thus accounted for. On the other hand, Mircoli considers that the use of tuberculin adds to the amount of toxine present in the blood, and may actually overthrow an existing balance of immunity and cause the patient to succumb to the disease, which otherwise he might have successfully resisted.

Most other observers who have tried Maragliano's serum for the treatment of tuberculosis have failed to produce any marked improvement by its means.

Marmorek's serum. — Marmorek[4] claims to have isolated from the *B. tuberculosis* a special toxine, differing from tuberculin (this he considers only a subsidiary substance which aids in the production of the true poison), and by the inoculation of this toxine in horses has prepared a serum which acts as a protective to animals against tubercular infection. Marmorek has used the serum in cases of pulmonary tuberculosis and tubercular pleurisy, and claims

[1] (?) 270. [2] (?) 664. [3] (?) 662. [4] See *Lancet*, Nov. 21, 1903, p. 1470.

good effects. He has also used it in tubercular meningitis, with some amelioration of symptoms, and thinks that it might prove curative of this condition if used early enough. The serum has been tried by a large number of observers with divergent results. Thus Ullmann[1] speaks of the serum as a specific remedy, superior to all other methods of treatment, and Monod,[2] Baer,[3] Schenker,[4] Roever[5] and Thieme,[6] to quote only a few recent writers, are favourably impressed with its value. Glaessner[7] and Hoffa[8] found the remedy useful in surgical tuberculosis (joints, bones, &c.), and van Huellen[9] in abscesses and peritonitis. On the other hand, Kraus[10] and Mann[11] found the serum useless in cases of pulmonary tuberculosis, and Stadelmann and[12] Benfey and Krokiewicz and Engländer[13] believe it to be actually harmful.

Bosanquet and French[14] observed a rise in the opsonin-index after rectal injections of the serum, but no improvement in the clinical features of their cases. In one instance in which the serum was administered subcutaneously a rapid fall in the opsonin-index occurred, and the patient appeared to be harmed rather than benefited. This would seem to suggest that the serum contains some toxic body allied to tuberculin, rather than an antitoxine.

The evidence is at present too conflicting to allow a

[1] *Wien. klin. Woch.*, 1906, No. 22.
[2] *Compt. Rend. Acad. Méd.*, Jan., 1907.
[3] *Münch. med. Woch.*, 1907, No. 24, p. 1670.
[4] *Münch. med. Woch.*, 1907, p. 2125.
[5] *Beitr. z. Klin. d. Tuberk.*, 1906, May 26.
[6] *Deut. med. Woch.*, 1908, No. 29.
[7] *Ibid.*, No. 16.
[8] *Berlin. klin. Woch.*, 1906, No. 44.
[9] *Deut. Zeitschr. f. Chir.*, 1906, Nos. 1-3.
[10] *Zeitschr. f. Tuberk.*, 1905, vii., Hft. 3.
[11] *Wien. klin. Woch.*, 1906, No. 42.
[12] *Berl. klin. Woch.*, 1906, 93.
[13] *Wien. klin. Woch.*, 1906, No. 11. (For further literature *see* Catz, *Progrés Médical*, 1908, No. 26.)
[14] *Brit. Med. Journ.*, 1907, Apr. 13.

definite conclusion to be formulated as to the value of the serum. It would seem to merit further trial in cases of surgical tuberculosis, but it has proved disappointing in pulmonary disease. Rectal administration of the serum (5 cc. daily) is to be preferred to the hypodermic method, but is disliked by patients. If the hypodermic method is employed, the doses should be smaller and the intervals between them longer.

Other serums.—Nicholls prepared an antitoxic serum by injecting goats with Koch's new tuberculin (T.R.). The injections were given subcutaneously in the neck, once a week, starting with doses of 0·0025 mg., and rising gradually to 15 mg. by the end of seven months. The serum thus prepared seemed to have some restraining power over the development of the disease in rabbits and guinea-pigs, but it could not be called curative.

Macfarland[1] injected an ass with tuberculin, and tried the serum obtained from it in fifteen cases of tuberculosis, but without definite results.

Di Capra[2] finds diphtherial antitoxine useful in pulmonary tuberculosis. He states that under its influence fever diminishes, cough is relieved, and appetite improves (*see also* Horse-Serum, p. 353).

On the whole, we have to confess that at present it has not been found possible to produce a serum which will influence tuberculosis to an extent at all comparable with the effects of antitoxine in diphtheria. This want of success is probably owing to our inability to prepare an adequately strong solution of toxines of the tubercle-bacillus. Much of the toxic matter of this organism seems to remain intracellular, and not to be given off into the culture-medium. The last word has not yet been said on the matter, and it is possible that more success will be obtained by further trials; but it is not in this direction that our hopes of combating the disease seem, at present, to point.

[1] *Journ. of the American Med. Assoc.*, 1897, ii. 359.
[2] *Giorn. Internaz. delle Sci. Med.*, 1903, No. 2.

ANTISTREPTOCOCCIC SERUM IN TUBERCULOSIS

It has already been pointed out that much of the destruction of the pulmonary tissue which takes place in cases of phthisis is due to a secondary infection of ulcerated surfaces by pyogenic bacteria, especially streptococci. The attempt has been made to combat these invaders by the use of antistreptococcic serum. Some results of this method of treatment are recorded by Bonney.[1] He selected cases in which large numbers of streptococci were to be found in the sputum. Other signs of infection with these organisms are to be seen in the occurrence of chills or irregular oscillation of the temperature, or in profuse sweating at night. Bonney used the serum in twenty-six cases, all of which were in an advanced stage of the disease and had failed to benefit by ordinary methods of treatment. As a result, three cases were apparently cured, and four others were set well on the way to recovery. Five cases improved distinctly, but not sufficiently to render recovery more than problematical; while eight others exhibited temporary amelioration of symptoms, but the course of the disease was not checked. In six cases no definite results were obtained. Menzer[2] has also obtained good results with his serum in these cases, and considers that the inflammatory reaction which occurs, due to its action, may have a beneficial effect on the tubercular lesions, besides combating the streptococci.

VACCINES IN TUBERCULOSIS

Gray[3] has used streptococcic and staphylococcic vaccines in cases of tuberculosis of bones, joints, and glands with advantage; the doses being 500–1,000 millions of staphylococci, and 100–250 millions of streptococci. We find this method of treatment extremely valuable in all forms of tubercular lesions which have become secondarily infected with pyogenic bacteria.

[1] *Med. News*, July 13, 1903.
[2] *Münch. med. Woch.*, Oct. 27, 1903, p. 1877.
[3] *Lancet*, 1906, i. 1099.

General considerations as to the nature of tuberculosis and immunity.—After the above discussion of the various methods which have been proposed for the prevention and cure of tuberculosis, it may be well to set forth a few general considerations which seem to have a bearing on the question of the production of immunity in this disease. In the first place, it is evident that tuberculosis differs from other infective diseases, as, for example, scarlet fever, in that one attack does not, as far as can be seen, confer immunity to subsequent infection. Indeed, it would rather appear that a person who has once developed a focus of tuberculosis is more liable to become the subject of subsequent outbreaks of tubercular trouble than one who has hitherto been free from infection. Maragliano, it is true, denies this, and holds that it is rare for one who has recovered from tubercular disease of a joint, for example, to develop tuberculosis of any other part later on in life. General experience, however, would show that those who have suffered from tuberculous glands in their youth are only too likely to become phthisical in after years, even if the glands have been removed or the affection has subsided. Hence it must be doubted whether any lasting immunity to the tubercular process can be conferred by artificial means, such as vaccination or the use of any form of tuberculin as a prophylactic. Of course, such an *a priori* argument would be at once overthrown if it could be shown that Behring's treatment in animals was really effective, but this still demands exact proof.

On similiar grounds it is hardly to be expected that it will prove to be possible to obtain a serum which will cure the disease. The tubercular process is very chronic, the bacteria growing very slowly and remaining alive in the body for a long time. Serums, whether antibacterial or antitoxic, are rapidly eliminated from the body. Hence to combat the disease it would be necessary to continue the injections of serum over a long period of time. The

difficulty and expense of such proceedings are at once apparent.

Further, the facts which we know as to the mode of resistance of the body to the disease do not give ground for much hope with regard to the attainment of artificial immunity. In the majority of cases tuberculosis is not of the nature of a general infection—those cases in which this is the case being just those which seem absolutely hopeless (acute general tuberculosis). It is a local infection, causing gradual destruction of tissue; resistance to which is carried out by means of the local reaction of the connective tissue round the infected area, leading to the formation of a protective capsule of fibrous material. It is true that the building-up of this protective wall seems to depend on the power possessed by the patient of resisting the poisons of the bacilli, and that, therefore, anything which tends to strengthen this resistance is of use; but it is not by any means the same process as that seen in recovery from a general infection, in which class of disease alone any real assistance has so far been obtained from serum-treatment. On these grounds we cannot reasonably be disappointed at the want of success which has so far attended attempts to apply serum-therapeutics to tuberculosis.

GENERAL CONCLUSIONS

(1) **Tuberculin.**—A. The *original tuberculin* (T.O.A.) affords a valuable means of diagnosis when inoculated cutaneously or injected hypodermically. It is not infallible, the margin of error amounting to about 10 per cent. Calmette's modification of the original tuberculin is particularly useful as a diagnostic agent. It should not be used indiscriminately, but only in cases in which other means of diagnosis have been tried and failed, and in which the question of the existence of tuberculosis is of immediate importance to the patient.

This form of tuberculin should not be used therapeutically, except perhaps in lupus.

B. The *new tuberculin* (T.R.) is of considerable value in the treatment of some cases of lupus vulgaris, especially those in which the disease has involved parts inaccessible to direct surgical or photo-therapeutic measures. It may do good also in some cases of laryngeal tuberculosis. The best results from its use are obtained in tuberculosis of the genito-urinary tract, of the peritoneum, of the eye, and of bones and joints. Early tuberculous adenitis also yields to tuberculin T.R., but enlarged glands of old standing do not undergo any marked alteration. It is contra-indicated in tubercular meningitis.

This form of tuberculin is of value in selected cases of pulmonary tuberculosis. It should be used along with ordinary treatment by open air and increased feeding, especially in cases which appear to be making little progress by the latter measures alone.

(2) **Agglutination-test.**—This method of diagnosis, as at present carried out, is complicated and unreliable. It is of no practical value for the diagnosis of phthisis. It may, perhaps, be of use for distinguishing between tubercular ascites and other collections of fluid in the abdomen.

(3) **Serum-treatment.** A. Treatment of tuberculosis by the serum of immunised animals is at present unsatisfactory. Maragliano's and Marmorek's serums may possibly be of some use, but the evidence is not conclusive.

B. In cases of pulmonary tuberculosis accompanied by the presence of large quantities of streptococci in the sputum, treatment with antistreptococcic serum appears to have given good results, and is certainly worthy of trial.

(4) **Protective vaccination.**—It is possible to render animals immune to tuberculosis by vaccination with attenuated bacilli. As to the announcement by Maragliano of a means of vaccinating human beings against the disease, it is too soon to form any judgment.

CHAPTER XIV

OTHER CONDITIONS TREATED BY ANTIBACTERIAL METHODS

DYSENTERY

Apart from affections caused by the *Entamœba dysenteriæ* (*E. histolytica*), the name dysentery is applied to cases of ulceration of the colon caused by a group of nearly allied organisms which share the title of *Bacillus dysenteriæ*. The exact relationship of the members of this group is as yet uncertain. Varieties are distinguished by the names of their discoverers, as the Shiga-Krüse bacillus, Flexner's bacillus, &c.

Mlle. Boïto[1] concludes from a study of agglutination experiments carried out with the various organisms that they fall into two groups—(1) that including the bacilli of Shiga, of Krüse, of Flexner (at New Haven), and others, which are agglutinated in dilutions of 1 : 400; (2) that including the bacilli found by Flexner at Manilla, by Krüse (asylums), and by some others, which have less agglutinative tendency. The question of the identity or diversity of the several organisms must be left at present unsettled.

Toxines.—Martin[2] finds that by growing Shiga's bacillus in peptone-broth a soluble poison is produced, which

[1] *Gaz. des Hôp.*, 1903, No. 97, p. 781, and No. 80, p. 809. A full bibliography of the subject is here given. *Cf.* Gay and Duval, *Univ. of Penn. Med. Bull.*, July-Aug., 1903, p. 177.

[2] *Thirty-first Ann. Rep. of L.G.B.*, 1901–2. *Supplement containing the Report of the Medical Officer*, 1903, pp. 398, 402. *Cf.* Kraüs & Doerr, "Das Dysenterie-toxin.," Jena, 1907.

causes lowering of the temperature of animals into which it is injected; the animals also suffered from diarrhœa and loss of weight. The most potent part of the poison of the bacillus is, however, intracellular, the effect of injection of the dead bodies of the organisms being similar in kind to that of the toxines, but much more pronounced. The toxine is apparently innocuous if given by the mouth, but if injected into a vein it causes the typical lesions of the disease.

Agglutination of B. dysenteriæ.—The *Bacillus dysenteriæ* is agglutinated by the serum of patients who have recovered from the disease, or of animals inoculated with cultures. The serum of convalescents was found by Shiga to clump the bacilli in dilutions of 1 : 20 or 1 : 30. This property may remain for a considerable time after recovery has taken place (*e.g.* 8 months), but the reaction does not appear at the beginning of the illness, so that it is of no importance for clinical diagnosis. Use has been made of it for the recognition of the bacillus, and for proving the identity of the varieties described by different authors. In mild cases the reaction may never occur. Krüse found that in cases of dysentery agglutination might occur in dilutions of 1 : 50, and even of 1 : 1,000, while normal individuals never showed the phenomenon in greater dilution than 1 : 20. Flexner found the reaction present in cases due to his bacillus, whereas it did not occur in cases of amœbic dysentery.

Nicolle and Cathoire[1] find that agglutination in dysenteric infection is feeble, and that the agglutinins appear to differ according to the variety of bacillus present. On the other hand, Dopter[2] finds that the bacteriolytic copula formed in response to injections of the bacilli themselves is the same from all the strains.

Serum-treatment.—Shiga inoculated a horse with his bacilli, and obtained from it a serum which acted bene-

[1] *Compt. Rend. Soc. Biol.*, 1906, No. 30.
[2] *Ann. Inst. Past.*, 1905, xix. 753.

ficially in cases of dysentery. He considered that by its use the mortality of the disease was reduced by nearly 50 per cent. Krüse considers that the serum prepared from his bacilli is bactericidal, not antitoxic. By the use of this remedy he obtained a fall of mortality from 10 to 8 per cent. These figures do not appear very striking. Shiga,[1] and also Coyne and Auche,[2] have prepared polyvalent serums by injection of bacilli belonging to all the different varieties. These serums are presumably antibacterial. Vaillard and Dopter[3] have prepared a serum by injection of both toxines and bacilli, and record good results (96 cases with 1 death): the dose used was 20–100 cc., repeated as necessary. Rosenthal[4] treated 157 cases with 7 deaths, a mortality of 4·5 per cent. as compared with 10–11 per cent. in other German hospitals; the doses used were large (20–120 cc.).

Lesage[5] prepared a serum by inoculation with his cocco-bacillus, which reduced the mortality in the cases which he observed by 50 per cent. Moreul and Rieux[6] also produced a serum by means of the variety of *B. coli* which they considered to be the causal agent in the cases which they examined; they found that it was both preventive and curative.

Vaccine-treatment.—In cases of chronic dysentery, rebellious to other treatment, Forster[7] found benefit to accrue from the use of a vaccine of dead organisms. He used cultures of Shiga's bacillus, heated to 60°–63° C., and suspended in salt-solution. The value of this treatment is confirmed by Stephens[8] and by Newman.[9]

[1] Abstr. *Centralbl. f. Bakt.*, 1908, xli. 742.
[2] *Compt. Rend. Soc. Biol.*, 1906, lx., No. 26.
[3] *Ann. Inst. Past.*, 1906, xx. 321; 1907, xxi. 241.
[4] *Deut. med. Woch.*, 1904, xxx., No. 19.
[5] *Bull. de la Soc. de Biologie*, 1902, p. 705.
[6] Quoted by Mlle. Boïto, *op. cit.*
[7] *Ind. Med. Gaz.*, 1907, p. 201.
[8] *Ibid.*, p. 375.
[9] *Lancet*, 1908, i. 1410.

INFANTILE ENTERITIS, OR SUMMER DIARRHŒA

The *Bacillus dysenteriæ* has been found in cases of the summer diarrhœa of infants, and is considered by some writers to be the cause of the disease.[1] Morgan[2] has isolated a bacillus, "No. 1," from some 63 per cent. of the cases which he investigated, and regards it as the causal agent. It is probable that more than one bacterial agent can give rise to the morbid conditions classed under this heading. Krüse obtained good results from serum-treatment in cases of dysentery in children, reducing the mortality from 15 to 5 per cent. Gay[3] considers that the prospect of serum-treatment in summer diarrhœa is very hopeful; but the reports of the Rockefeller Institute on the use of antidysenteric serum in this affection are not encouraging.[4]

COLITIS

Membranous, mucous and ulcerative colitis are terms employed to denote a condition or group of conditions which some observers consider to be identical with bacillary dysentery, especially the cases which occur in asylums for the insane. Sporadic cases of long standing have been found by Hale White and Eyre[5] to be amenable to treatment with vaccines prepared from *B. coli communis* and coli-form bacilli present in the intestine and fæces of patients.

YELLOW FEVER

Ætiology.—The causation of yellow fever is at present unsettled. Sanarelli[6] isolated a bacillus to which he gave

[1] *See* Martha Wollestein, "The Dysentery-bacillus in a Series of Cases of Infantile Diarrhœa," *Journ. of Med. Research*, Aug., 1903, p. 11.

[2] *Proc. Roy. Soc. Med.*, March, 1909.

[3] *Univ. of Penn. Med. Bull.*, Nov., 1902.

[4] See *Brit. Med. Jour.*, 1904, i. 1653.

[5] *Lancet*, 1909, i. 1586.

[6] *Ann. de l'Inst. Pasteur*, 1897, No. 6, p. 433.

the name of *Bacillus icteroides*, and which he believes to be the pathogenic agent. The claims of this organism, however, have not been supported by other observers. Parker, Beyer and Pothier [1] have found a protozoan organism which they named *Myxococcidium stegomyiæ*, and which is possibly the cause of the disease. This coccidium is found in the bodies of gnats which have sucked the blood of patients suffering from yellow fever. It is now practically proved that the disease is transmitted by the bites of the variety of gnat called *Stegomyia fasciata*, and Findlay [2] suggests that this insect is the principal host of the parasite of yellow fever, which only passes a subordinate stage of its existence in human beings. Its life-cycle would thus be just the opposite of that of the organism of malaria, which has man for its definitive host. It is, however, quite possible that the true cause of the disease still remains to be discovered.

Serum-treatment. — Sanarelli [3] prepared an "anti-amarillic" serum [4] by inoculating a horse with his bacilli, and used it in cases of the disease, apparently with good effect. He reports that the injection is followed by a febrile reaction, which in turn is succeeded by remission of the symptoms. Among eight cases in which he used small doses of the serum there were two deaths and six recoveries; while of fourteen severe cases in which larger quantities were employed ten recovered.

Prophylactic injections of the serum were used in the case of an outbreak which had occurred in a jail, and after the injections were carried out no more cases of the disease were met with.

Agramonte [5] has tried the *serum of convalescents* in

[1] *United States Public Health and Marine Hospital Service* (*Yellow Fever Bulletin*, No. 13), 1903.

[2] *Revista de Medicina Tropical*, 1903, No. 4, p. 49.

[3] *Ann. de l'Inst. Pasteur*, 1898, p. 348.

[4] Amarillic, from the Spanish name of the disease, "Fiebre amarilla."

[5] Quoted by Fitzpatrick, *Journ. of the American Med. Assoc.*, April 14, 1900, p. 905.

yellow fever, and thinks that good effects are produced. Other observers have not been equally successful.

Toxines of B. icteroides.—Baker[1] injected toxines derived from *Bacillus icteroides* into patients, and found that they produced the typical phenomena of a rising pulse and falling temperature (Faget's pulse and temperature), which are peculiar to this disease.

Vaccination with the *Bacillus icteroides* in accordance with Haffkine's method is capable of protecting animals against infection with this organism.

WHOOPING-COUGH

It is almost certain that whooping-cough is due to some infective micro-organism, but this agent has not been definitely identified. Various organisms have been claimed by different observers as the causal agent. That described by Jochmann, Bordet and others, and belonging to the influenza group of hæmophilic bacteria, possesses the strongest claims to recognition.

Specific serums.—Elena Manicatide[2] obtained pure cultures of a peculiar bacillus from the sputum of patients, and with them inoculated a sheep and a horse. The serum obtained from these animals appeared to exert a favourable influence on the disease, the number of attacks diminishing under the treatment, and convalescence being more rapid.

Leuriaux[3] found in cases of whooping-cough a short, thick bacillus, almost as broad as it was long. It was motile and aërobic, growing well on gelatine and other laboratory media; and it retained the colour when treated by Gram's method. He inoculated rabbits with this organism, which produced death if it was given intravenously, while if it was given subcutaneously a local

[1] See *Journ. of the American Med. Assoc.*, April 14, 1900.

[2] *Spitalul*, 1902, No. 6 (abstr. in *Centralbl. f. inn. Med.*, 1903, p. 199).

[3] *La Semaine Méd.*, 1902, p. 233.

abscess was formed. Convulsive movements of the diaphragm were seen in some instances, which may be analogous to the convulsive seizures of pertussis. Leuriaux inoculated a horse with the organisms, and treated cases of whooping-cough with its serum. He considers that the effects produced were good, cure being brought about in six to eight days. He employed doses of 5 cc., and advises the early administration of the remedy.

It is noteworthy that these two observers agree in finding a specific bacillus and in obtaining good results by serum-treatment. It is impossible to know whether the organism isolated was the same in both cases. Much further experimental evidence is necessary, both as to the specificity of the bacillus and as to the efficacy of the serum, before we can conclude that a true remedy has been discovered for this malady. The course of the disease is so irregular, and the difficulty of judging of the value of any treatment is so great, that a considerable degree of scepticism is justifiable in the case of any new "cure" which is brought forward.

Silvestri[1] used injections of the *serum of convalescents* (15–20 cc.) in seven cases of whooping-cough, all of which were apparently relieved.

Non-specific treatment.—Indica[2] and other observers speak favourably of the use of diphtherial antitoxine in whooping-cough. Indica treated eight cases with this serum, which he considers to act by stimulating leucocytosis and increasing the resistance of the patient.

Porchi[3] thinks that vaccination (vaccinia) is both prophylactic and curative.

LEPROSY

Ætiology.—The *Bacillus leprœ* was discovered by Hansen in 1886. Attempts to cultivate it artificially have

[1] *Gaz. degli Ospedali*, 1903, No. 114.
[2] *Ibid.*, 1900, xxi. 968.
[3] *Ibid.*, 1903, No. 114.

generally failed, but one or two successful cultures have been reported. The lower animals appear to be insusceptible to this organism.

Serum-treatment.—Carrasquilla[1] endeavoured to prepare an antidotal serum by injecting the blood of lepers into asses and young horses. The resulting serum is probably quite inert.

Attempts have been made to treat the disease with *antivenene*, and good results are claimed for this method by Dyer[2] (five cases; two cured, two improved). Injections of *tuberculin* have also been tried, and have apparently resulted in some softening of leprous nodules, but no permanent good has been effected. Abraham[3] states that "fresh nodules crop up, and the patients are generally no better off after treatment than before." The disease runs a very chronic course, with intervals of improvement or cessation of advance. Hence many remedies have been advocated as cures, the natural remissions being attributed to their action. There is, unfortunately, no greater reason to think that any serum is a reliable remedy, than to believe in the methods of drug-treatment which have been advocated.

Vaccine-treatment.—Deycke and Reschad[4] isolated a streptothrix from cases of leprosy and used it as a vaccine: they observed good results. They have also separated from the organisms a fatty substance to which they give the name of *Nastin*, and which they use in the form of an oily solution for injection. They suggest that it might also be of use in tuberculosis.

Kupffer[5] reports very favourably on the results obtained from the use of Nastin, although in many of his cases chaulmoogra oil was also administered.

[1] *Deut. med. Woch.*, 1897.

[2] *New Orleans Med. and Surg. Journ.*, Oct., 1897.

[3] Art. "Leprosy," in Allbutt's "System of Medicine," vol. ii., p. 78.

[4] *Deut. med. Woch.*, 1907, 89.

[5] *Lepra Bibliotheca Internat.*, 1909, viii., Part 3.

STREPTOTRICHOSIS

Wynn [1] has recorded a case of actinomycosis of the lung successfully treated with a vaccine. The patient was a boy of 14 years, and the dose used was 0·001 mg. of the solid substance of the organism isolated from the pus and grown on agar ("actinomycotin").

RELAPSING FEVER

Ætiology.—The causal agent of relapsing fever was discovered by Obermeier, and is named after him the *Spirillum or Spirochæte obermeieri.*

Agglutination and bacteriolysis. — Sawtschenko and Melkich [2] found that the serum of patients towards the end of the disease had an agglutinative power on the organisms, and that it also contained a copula or immune body which was capable of producing destruction of the bacteria within the leucocytes. They hold that the action of this body is to transform the negative chemiotactic effect of the organisms upon the leucocytes into positive chemiotaxis (*i.e.* opsonising). There was no free complement in the blood-plasma, so that no bacteriolysis took place outside the cells. Heating the serum led to loss of its bacteriolytic property, but not of its agglutinins.

Hodlmoser [3] finds the serum of convalescents to be bacteriolytic rather than agglutinative.

Serum-treatment.—Löwenthal [4] has produced a bactericidal serum by inoculation of horses with the spirilla, and good results are claimed as the result of its use.

Dobrosrakow [5] also immunised horses with the defibrin-

[1] *Brit. Med. Journ.*, 1908, i. 554.

[2] *Ann. de l'Inst. Pasteur*, 1901, p. 207. The bactericidal power of the blood of the convalescents was first shown by Gabritschewsky. *Ann. de l'Inst. Pasteur*, 1896.

[3] *Zeitsch. f. Heilk.*, xxvi., Hft. 11.

[4] *Deut. med. Woch.*, 1898.

[5] Abstr. *Centralbl. f. inn. Med.*, 1907, p. 1039.

ated blood of patients suffering from relapsing fever, and used their serum for treatment, with apparent benefit.

PELLAGRA

This disease, characterised by weakness of the limbs, ending in paralysis, with peculiar mental symptoms, is said to be due to the use of diseased maize as food. The poison contained in this substance can be extracted, and is capable of causing the death of animals. Babes and Manicatide[1] found that if the extract was injected along with blood-serum from normal persons, the fatal effect was not prevented ; but that if serum from those convalescent from the disease were used, it protected the animals from the effects of the poison. An antitoxic substance is therefore contained in the serum of convalescents.

SYPHILIS

Ætiology.—It is now generally believed that the causal organism of syphilis is the spirochæte discovered by Schaudinn, and now known as the *Treponema pallidum* (*Spirochæte pallida*, *Spironema pallidum*).

Serum-therapeutics.[2]—Since the lower animals, with the exception of the ape, are immune to syphilis, various attempts have been made to influence the course of the disease by injections of the serum of animals. Richet and Héricourt injected the serum of dogs ; and other writers have recorded experiments with the serum of other species (lambs, Tommasoli ; horse, Kamnberg ; cattle, Kollmann). No satisfactory results have been obtained by this means.

Injection of syphilitic blood into animals, and inoculation with other products of the disease (hydrocele and ascitic fluid ; chancres), have also been tried, the serum being then drawn off, and used therapeutically (Mazza, Gilbert and

[1] *La Semaine Méd.*, 1900, p. 279.

[2] For the literature on this subject, *see* Fouquet, *Gaz. des Hôp.*, Oct. 10, 1903, p. 1153 ; also Lane, *Practitioner*, July, 1904.

Fournier). Improvement is stated to have ensued in some of the patients thus treated.

Risso and Cipollina[1] used a serum derived from dogs and asses, in doses of 2–5 cc., and saw good effects produced in all stages of the malady.

Query,[2] who believes that the treponema is a stage in the development of a bacillus which he isolated, made use of this for immunising animals, and thence prepared a serum, which Hallopeau[3] believes to be of value in treatment.

The serum of patients in the tertiary stage of the disease, and that of congenitally-syphilitic infants, has also been employed. Good results are stated to have been attained in some cases by this means (Gilbert and Fournier, Boeck, Moore, &c.); but it is evident that such a mode of treatment could never be generally used.

Serum-reaction in syphilis.—The presence of specific antibodies in the blood-serum and cerebro-spinal fluid of patients infected with syphilis can be demonstrated by modification of the Gengou-Bordet reaction (Fixation of Complement, p. 31) described by Wassermann and Plaut and known as Wassermann's test.[4] In this test a watery extract of syphilitic fœtal liver[5] is used as the antigen to fix the syphilitic immune body present in the serum, and the hæmolytic system (*see* page 7) is arranged as follows:

The serum from the patient previously inactivated by heating (therefore only containing immune body), mixed with liver-extract and a small quantity of fresh serum (to provide the complement) is incubated at 37° C. for one hour; to it are then added sensitised red cells (that is to say, red cells mixed with their appropriate, but inactivated, hæmolytic serum) and the mixture is again incubated

[1] *Rif. med.*, Nov. 30, 1904; Mar. 18, 1905.

[2] *Compt. Rend. Soc. Biol.*, Mar. 9, 1907.

[3] *Ibid.*, Dec. 21, 1907.

[4] *Berlin. klin. Woch.*, 1907, Nos. 50 and 51.

[5] Henderson Smith substitutes a watery dilution of alcoholic extract of normal guinea-pig's heart, which apparently contains the necessary lipoid.

for two hours at 37° C., removed to an ice-chest, and allowed to stand for 12 to 18 hours before examination. A similar hæmolytic system is put up at the same time to act as a control, in which the suspected serum is replaced by that from a normal individual. If syphilitic antibodies are present in the suspected serum, no hæmolysis will take place, as the complement required by the sensitised red cells for this reaction to occur will have been already utilised and bound by the syphilitic immune body to the lipoid of the liver-extract. This constitutes a positive reaction. In the absence of immune body from the suspected serum, however, complete hæmolysis will take place and a negative reaction is recorded.

Plaut[1] obtained a positive reaction in 80 per cent. of cases of undoubted syphilis. Levaditi and Yamanouchi[2] found that the cerebro-spinal fluid only yields positive results when the nervous system is involved.

MICROCOCCUS MELITENSIS SEPTICÆMIA (MALTA FEVER)

Ætiology. This disease is due to a minute oval coccus, the *Micrococcus melitensis*, discovered by Bruce in 1888.

Agglutination.—The micrococci are agglutinated by the serum of sufferers from the fever. The reaction may appear as early as the fourth day, seldom later than the sixth, and occurs in considerable dilutions (1 : 100, 1 : 1,000, up to 1 : 500,000).[3] This reaction is very valuable as a means of diagnosis, but certain precautions must be observed in carrying it out. The cultivation of *M. melitensis* must be one recently isolated, or one recently passed through the body of an animal ; the reaction must be positive in dilutions of 1 : 100, or at least 1 : 50 ; and the frequent occurrence of inhibition-zones must be remembered, for it is quite common to observe a positive reaction in dilutions of 1 : 50

[1] *Zentralbl. f. Nervenheilk*, 1908 ; Hft. 8.

[2] *Compt. Rend. Soc. Biol.*, lxii., 1907, p. 240 : lxiv., 1908, p. 349.

[3] Eyre, Milroy Lectures, *Lancet*, 1908.

or 1 : 100 of a serum which fails to agglutinate the coccus in dilutions of 1 : 10 and 1 : 20.

Serum-treatment.—Wright and Semple [1] have treated one case of this disease with a curative serum. The mode of preparation of this is not stated, nor can trial in a single case afford any trustworthy evidence of the value of the remedy.

Eyre [2] also prepared a serum from a horse, which agglutinated in dilutions of 1 : 3,000 to 1 : 5,000, and had some prophylactic power ; but it did not prove very effective as a remedy for the disease.

Vaccine-treatment.—Reid [3] used therapeutic inoculations, controlled by estimations of the opsonin-index, as a mode of treatment, with good results. Bassett-Smith [4] also prepared a vaccine by heating agar-cultures of the micrococcus to 60° C. for half an hour, but did not find it efficacious in the treatment of the disease.

Eyre [5] employed similar vaccines for *prophylaxis*, and considered that some protection was thereby afforded.

AFFECTIONS DUE TO BACILLUS COLI COMMUNIS

The *Bacillus coli communis* is a normal inhabitant of the human intestine. Under certain circumstances, such as constriction of the gut, injury to the peritoneum, perforation of the intestine, &c., it is capable of giving rise to serious symptoms ; it may also be found in suppurative conditions, such as pyelitis and cystitis, otitis media, &c., in all of which it acts as a pyogenic organism.

B. coli may also give rise to a true septicæmia, and is frequently associated with *Streptococcus longus* in the causation of puerperal septicæmia, thus explaining the occasional failure of antistreptococcic serum to check the course of that infection.

[1] *Lancet*, 1899, i. 1024.
[2] *Rep. Medit. Fever Commiss.*, 1907, Parts v.–vi.
[3] *Ann. Rep. Sanit. Com. with the Gov. of India for* 1905, p. 153.
[4] *Journ. of Hyg.*, 1907, vii. 115.
[5] *Rep. Medit. Fever Commiss.*, Part vi.

Carega [1] has isolated two toxic substances from broth-cultures of these bacilli—a nuclein and a nucleo-albumin: to the latter, no antibody is apparently formed in injected animals.

Serum-treatment.—Attempts to prepare an antitoxic or bactericidal serum against this organism have not been successful in the hands of Albarran and Mozer [2]; but anticoli serums are still on the market.

Vaccine-treatment has proved of great value. Thus Wright [3] caused the closure of a sinus left after operation by means of a vaccine of the organism, and this procedure was also used by Hawkins and Corner [4] in a case of infection following laparotomy for appendicitis. Gray [5] has also used coli-vaccine with success in cases of pyelitis; and Hicks [6] reports favourably on its use in the treatment of the pyelitis of pregnancy. Good results are reported in two cases of cholecystitis, the dose of vaccine being 100 to 200 millions of dead bacilli.[7] We have found the treatment of great value in cases similar to the above; also in cystitis, subdiaphragmatic abscess, ulcerative colitis, &c. The vaccine must be prepared from the patient's own organism, for in coli-infections stock vaccines are useless. The initial dose is usually 2·5 to 5 millions, repeated in from two to three days. Subsequent doses are generally given at intervals of about a week in increasing quantities up to a maximum of 50 millions.

AFFECTIONS DUE TO BACILLUS PYOCYANEUS

This pathogenic organism is called also the bacillus of blue pus. MacIntyre [8] found that the bodies of the bacilli contained an intracellular toxine and a hæmolysin.

[1] *Centralbl. f. Bakt.*, 1903, xxxiv., No. 4.
[2] *Journ. of the Amer. Med. Assoc.*, Jan. 17, 1899.
[3] *Trans. Path. Soc. London*, 1906.
[4] *See* p. 230.
[5] *Lancet*, 1906, i. 1102.
[6] *Brit. Med. Journ.*, 1909, i. 203.
[7] Wright and Reid, *Brit. Med. Journ.*, 1906, i. 143.
[8] *Journ. Amer. Med. Assoc.*, April, 1904, p. 1074.

Wassermann obtained both an antitoxic and a bactericidal serum by injection respectively of the toxines and of the bacillus itself into animals. These serums had both a protective and a curative action on animals.

We have found *vaccine-treatment*, in doses of 50 and 100 millions, of value in cases of osteomyelitis and suppurating sinus following laparotomy for ovarian tumour.

An extract of the bacilli was prepared by Emmerich and Löw [1] by autolysis and is called "pyocyanase." It seems to act as a bactericidal agent. It has been used by Hofbauer [2] in cases of vaginal gonorrhœa, with only transitory benefit, and by Escherich [3] as a local disinfectant in nasal catarrh due to *Micrococcus catarrhalis* and in cerebro-spinal meningitis; in the former affection it was very successful, but in the latter its value was not definitely proved. It has also been used as a local application to the false membrane in diphtheria.

AFFECTIONS DUE TO STAPHYLOCOCCI

Staphylococci are probably always present on the skin of mankind. They are capable of giving rise to suppuration in favourable circumstances. Several varieties are generally described, *Staphylococcus pyogenes aureus*, *citreus*, and *albus* being the commonest.

Toxines of staphylococci.—Denys found that by growing the staphylococci in fluid derived from serous exudates he could obtain a toxic substance which had the property of dissolving the leucocytes of the blood. The leucocytes, when treated with this substance, to which he gave the name of "leucocidin," first became transparent, with clearly defined nuclei, and finally were broken up and destroyed. The importance of this toxine in the parasitic life of the bacteria is evident, since it is largely by means of the eucocytes that the infected individual resists the invading

[1] *Zeitschr. f. Hyg.*, 1899, xxxi. 1; 1901, xxxvi. 9.
[2] *Centralbl. f. Gynäk.*, 1908, No. 6.
[3] *Wien. klin. Woch.*, 1906, xix. 751.

organisms. It is in this sense that we must look upon the formation of local collections of leucocytes (abscesses) at the points where the cocci settle. Leucocidin prepared from cultures in rabbits does not appear to have the power of dissolving human leucocytes. Other poisonous substances which have the property of exciting suppuration have been obtained from the bodies of staphylococci. To one of these Leber gave the name of "phlogosin."

Serum-treatment.—Several attempts have been made to obtain an antistaphylococcic serum. Antibodies can be obtained to the various poisons formed by the cocci, and rabbits can be immunised against the organisms. A serum capable of protecting these animals against several times the minimal lethal dose of the cocci has been prepared. It is doubtful if any good effects are to be looked for in man. The fact that the leucocidin which is active for rabbits' cells does not affect human leucocytes suggests that both the toxines and their antibodies may be different in different surroundings, so that animals' serum would be unlikely to have a curative action in human disease. Vicquerart and Doyen have prepared antistaphylococcic serums for purposes of treatment, but their value is doubtful. Moritz[1] tried antistaphylococcic serum in six cases of acute endocarditis (5 cc. doses), and was favourably impressed by the apparent results.

Agglutination.—Staphylococci are agglutinated by immune serum, and this reaction is suggested by Bruck, Michaelis and Schultze[2] as a means of diagnosis.

Vaccination.—Wright[3] inaugurated the use of dead cultures of the organisms as a vaccine in cases of acne and sycosis, with benefit.

Stock vaccines are exceedingly useful in staphylococcic infections, though distinctly inferior in efficiency to autogenous vaccines. Least useful of all are "mixed" vaccines

[1] *St. Petersb. med. Woch.*, 1898, No. 19.

[2] *Zeitschr. f. Hyg.*, 1905, l. 144.

[3] *Lancet*, March 29, 1902; *Brit. Med. Journ.*, 1904, i. 1075.

of *Staphylococcus albus* and *aureus*. In every case the particular type of staphylococcus responsible for the lesion, whether aureus or albus, must be determined, and a corresponding vaccine be employed. Furunculosis, solitary boils and carbuncles, sycosis, periostitis and osteomyelitis, septicæmia and pyæmia are usually due to *Staphylococcus aureus*; acne indurata to *Staphylococcus albus*. Generally speaking, the control afforded by the opsonic index can be dispensed with, and the injections given in accordance with the clinical phenomena. For example, in a case of furunculosis the immediate result of an injection of vaccine is the appearance of a fresh crop of boils; the majority of these, however, quickly abort, and the condition improves. The appearance of another set of boils is the signal for a further injection of vaccine. The size of the dose is a matter for careful consideration, and can usually be gauged from clinical observations following the initial tentative dose. In treating boils, &c., if the infecting organism is diagnosed in the early stages, a dose of 10, 25 or 50 millions may cause rapid resolution. If, on the other hand, a chronic indurated focus of long duration is present, it is often advisable to administer a large dose of 250 or 500 millions, in order temporarily to depress the patient's resistance, and at the same time to hasten the breaking down of the abscess by fomentations, &c., as a preliminary to evacuating the pus.

An average initial dose would be 50 millions in a localised suppuration, and a dose one-tenth of that in a generalised infection. When stock vaccines are employed, doses from twice to four times as large should be administered to ensure comparable results.

Whitfield[1] notes that fresh boils may occur during the use of the vaccine. Gilchrist[2] has cured bullous erythema with vaccine. He states that affections due to *Staphylococcus aureus* may be cured by the use of an inoculation of *Staphylococcus albus*.

[1] *Lancet*, 1908, ii. 731. [2] *Ibid.*

NASAL CATARRH

Allen[1] states that "a cold in the head" or coryza may be due either to Friedländer's bacillus, to the *Micrococcus catarrhalis* or to *B. septus*. Vaccine-treatment may cure chronic colds, and prevent recurrence in persons who are prone to them. He uses a vaccine consisting of 150 millions of *M. catarrhalis* in cases due to this organism (which may also be responsible for tracheal catarrh). It is advisable to prepare a vaccine from the organism derived from the individual patient. We are, however, convinced that the dose above recommended is too large, and rarely give more than 25, or at the outside 50, millions.

PYORRHŒA ALVEOLARIS

Pyorrhœa alveolaris or Riggs' disease is an acute or chronic suppuration of the alveolar processes, often associated with arthritic pains. It is apparently due sometimes to one organism, sometimes to another, and is remarkably amenable to treatment by vaccines. In a series of 24 cases we have observed *Streptococcus pyogenes* 7 times, *M. catarrhalis* 8 times, a mixture of these two cocci 6 times, and the pneumococcus 3 times. Small doses, 5 or 10 millions of the appropriate vaccine, injected every six to eight days, give the best results, and treatment may need to be prolonged for from three to six months. Goadby[2] has used a vaccine of *Staphylococcus aureus* successfully in two cases due to this organism.

LEUCHÆMIA

This disease, characterised by anæmia (defect of red corpuscles), with immense increase in the number of white corpuscles, by enlargement of the spleen and of the lymphatic glands, and sometimes by the appearance of masses of lymphoid tissue in the internal organs, is of uncertain origin.

[1] *Lancet*, 1909, ii. 1589, 1659.

[2] *Brit. Med. Journ.*, 1908, ii. 477.

It is allied on the one hand to the group of blood-diseases, including pernicious anæmia and splenic anæmia, and on the other hand to Hodgkin's disease (lymphadenoma) and the various tumours.

Injections of tuberculin have been used in a few cases, apparently with some diminution in the number of leucocytes (Henck, Beitzke). Rolleston has also administered antistreptococcic serum in one case with similar effect, but the result on the general condition of the patient was inconclusive.[1]

Leucatello and Malon[2] have used a leucolytic serum obtained by injecting animals (rabbits, sheep) with leucocytes. They record good effects, seen in diminished number of leucocytes and shrinking of the spleen.

PAROXYSMAL HÆMOGLOBINURIA

Widal and Rostanne[3] believe that in this disease hæmolysis occurs owing to the lack of an "*antisensibilitrice*" (anticopula) which is present in the blood of normal persons. They tried as a remedy the serum of a rabbit, which had been injected with human blood—with a view to increasing the formation of this body—and believed that good resulted from this mode of treatment. Eason[4] finds that in these patients there is present a copula, which induces hæmolysis by uniting with the appropriate complement under the influence of *cold*—a peculiar condition, since in other cases cold inhibits this action.

GENERAL PARALYSIS OF THE INSANE

Bruce[5] maintains that this disease is due to poisoning by bacterial toxines. He was at first inclined to regard it

[1] *See* Susmann, "Leuchæmia and Tuberculosis," *Practitioner*, 1903, ii. 541.

[2] *Gaz. degli Ospedali*, 1903, No. 11.

[3] *Compt. Rend. Soc. Biol.*, 1905, lviii. 321, 372.

[4] *Scottish Med. and Surg. Journ.*, May, 1906.

[5] *Ibid.*, June, 1903, p. 481.

as due to *B. coli communis*, since he found that the serum of patients suffering from general paralysis agglutinated these organisms; but he now considers this infection to be secondary.

Injection of serum derived from cases of the disease which were in a state of remission seemed to have a beneficial effect on patients who were in an acute phase of the malady. Vaccination with *Streptococcus pyogenes* also seemed to produce temporary amelioration in two cases in which the procedure was followed by a reaction. These patients, however, rapidly became immunised, and ceased to react. In one case, which showed no reaction from the first, no improvement was observed as a result of vaccination. The good effects may perhaps be connected with increased leucocytosis, since leucopenia is one of the features of the disease; and an attack of any febrile disease may produce temporary improvement in general paralytics.

Ford Robertson,[1] on what would appear to be insufficient evidence, considered that the toxines of the *B. diphtheriæ* played an important part in the production of the paralytic symptoms of the disease. He treated six cases (in various stages) with diphtherial antitoxine, given in doses of 2,000 units, but did not observe any material benefit. He then extended his observations, and described two varieties of diphtheroid bacilli,[2] which he regarded as of prime ætiological importance; but his views have not been confirmed.

EPILEPSY

Ceni[3] has injected epileptic patients with serum derived from other sufferers from the same disease, and obtained contradictory results. He has also used the serum of rabbits which had received injections of the serum of patients suffering from epilepsy. The injection of this (rabbit's) serum into epileptic subjects was followed by a

[1] *Rev. of Neurol. and Psychiat.*, May, 1903.
[2] *Journ. Mental Science*, 1907, liii. 590, 750.
[3] *Neurologisches Centralbl.*, April 16, 1903, p. 338.

febrile reaction, and sometimes by the occurrence of status epilepticus. Ceni considers that the blood of epileptics contains "auto-cytotoxines," consisting of copula and complement, while the serum of the rabbits contained excess of copula. No practical treatment has been founded upon Ceni's observations, which still need confirmation.

HYDATID DISEASE

Fleig and Lisbonne[1] found that the fluid contained in a hydatid cyst gave a precipitate when mixed with the serum of a patient suffering from the disease. This observation is confirmed by Welsh and Chapman,[2] who use this reaction as a means of diagnosis: 12 drops of serum should be added to 1 cc. of the fluid. Different specimens of hydatid fluid vary in their power of giving the reaction, and careful selection of a suitable sample must be made, if the phenomenon is to be used as a test for the presence of the parasite. If the reaction persists after operative removal of a cyst, it is probable that other cysts remain behind; but disappearance of the reaction does not prove the disease to be cured. Weinberg[3] employed a "fixation of complement" reaction in diagnosis. The antigen used is hydatid fluid, and the test is performed as in the syphilis reaction (p. 317). A correct diagnosis was based on a positive reaction in 26 out of 27 cases subsequently operated upon.

HAY-FEVER

Ætiology.—Not only the peculiar specific affection connected with the pollen of certain grasses, but also nervous conditions, of an asthmatic type, occurring in the summer months, are probably often included under the name of Hay-fever. Dunbar[4] has satisfactorily proved that the pollen of rye and other grasses is responsible for true hay-fever. The

[1] *Compt. Rend. Soc. Biol.*, 1907, lxii. 1198. [2] *Lancet*, 1908, p. 1338.
[3] *Ann. de l'Inst. Pasteur*, 1909, xxiii. 473.
[4] "Zur Ursache und Specifischen Heilung des Heufiebers," 1903, and *Deutsch. med. Woch.*, 1903, No. 9.

pollen-grains contain a soluble toxine which is capable of affecting susceptible persons, whereas normally-constituted individuals suffer no ill effects from it. Dunbar's experiments were as follows: He took the pollen of rye and applied it to the nostrils of a certain number of persons who suffered periodically from hay-fever, and also to those of others who had not experienced the disease. In the former definite symptoms of coryza and irritation of the nasal mucous membrane were produced; while the latter did not exhibit any ill effects. Similar results were produced in susceptible persons by applying the pollen to the conjunctivæ. That the symptoms were not due to the mechanical effects of the grains of pollen was proved by control experiments with materials derived from other plants, including those varieties which have pollen-grains of a prickly form. Of two persons who were placed in a room and caused to blow into a vessel containing the toxic pollen, one who was susceptible to hay-fever exhibited symptoms of bronchitis and asthma, while the other was unaffected.

The soluble nature of the toxine was demonstrated by making aqueous and ethereal extracts of the pollen and applying them in the same way to patients. Symptoms of hay-fever were invariably produced in susceptible persons. If the pollen is kept for any length of time, so that it becomes dry, it no longer induces symptoms; but if the dried grains are broken up, the toxic effects are again manifested. In ordinary cases it seems that the poison is dissolved out by the tears and nasal secretion, and is thus enabled to act. Dunbar found that symptoms of irritation were produced by application of the toxic pollen to other mucous surfaces beside those of the eye and nose, *e.g.* by application to the anus. If a solution of the poison is injected hypodermically, very similar effects are produced to those seen after its local application. Thus, a medical man, subject to attacks of hay-fever, received an injection hypodermically in the arm. He first felt giddy, and in a

quarter of an hour was seized with sneezing, cough, lachrymation, soreness of the throat, and dyspnœa. The face swelled and the voice became hoarse; there was respiratory stridor, and by laryngoscopical examination the vocal cords were seen to be congested. The frequency of both pulse and respiration was increased. A cutaneous eruption ensued, of an urticarial nature, with great itching; the arm in which the injection was given became markedly swollen. A second subject, who did not suffer from hay-fever, exhibited no ill effects from a similar injection.

Antitoxine.—Dunbar proceeded to attempt the manufacture of an antitoxine to counteract the toxine contained in the pollen. He injected the latter into rabbits, and used their serum for experiments. He found that the serum obtained from these animals was capable of neutralising the toxine and protecting susceptible persons from its effects. Thus, if some of the serum were added to the toxine, the mixture could be introduced into the eye of a susceptible person without any ill effects. Serum from a normal rabbit had no protective action.

Several other writers confirm Dunbar's results. Semon[1] has tried the serum, and finds that definite effects are produced. It cannot be described as curative of the disease, but considerable improvement is produced by its use. The effects differ considerably in different cases. If the serum be applied to the nose and eyes, when the first symptoms are experienced which are known by the patients to portend an attack of hay-fever, the threatened access may be aborted. In some cases the remedy unaccountably fails. The subjective relief produced in patients is often greater than the diminution in the objective signs of the disease. The duration of the relief afforded is not long, and repeated instillations of the antitoxine are required. Subcutaneous injections are not advisable, as the local œdema produced is considerable, and the amount of protection gained is uncertain.

[1] *Brit. Med. Journ.*, 1903, ii. 123, 220.

M'Bride[1] also is favourably disposed towards the use of the serum. He concludes that Dunbar has definitely succeeded in isolating the toxine of the disease and in producing an antitoxine capable of neutralising it. It is doubtful, however, whether it is the pollen of grasses alone which is responsible for the disease, and therefore whether we possess in the serum an antidote to all forms of hay-fever. Some persons suffer from a similar catarrhal condition if they are exposed to the smell of horses or cats, and it is evident that the serum prepared from pollen cannot be expected to act beneficially in such cases. Knight[2] collected 219 cases in which the serum was used; of these 114 show great improvement and 66 slighter benefit.

Thost[3] also confirms Dunbar's results, but points out that in all cases of hay-fever there is a certain element of nerve-weakness, which cannot be influenced by the serum; while the cases which are complicated by morbid local conditions will need appropriate treatment for these, as well as the specific remedy.

Weichardt[4] points out that in many cases the serum may do good at first, but is afterwards not tolerated. We have seen a certain number of instances in which it seemed to afford relief, but seldom, if ever, a result that could be described as a cure. Nevertheless the remedy is well worthy of trial in severe cases of the affection.

Autumn catarrh.—In the United States of America there is a form of hay-fever, known as "autumn catarrh," which appears in the autumn, at a time when there is no pollen from rye in the air. It seems necessary to suppose that these cases are due to a different cause. Dunbar[5] has investigated some of the plants which might be responsible

[1] *Edinburgh Med. Journ.*, 1903, ii. 7.

[2] *Med. Record*, Mar. 10, 1906.

[3] *Munch. med. Woch.*, June 9, 1903. *Cf.* Lübbert, *Therap. Monatsh.*, Dec., 1904.

[4] Abstr. *Centralbl. f. Bakt.*, 1906, xxxviii. 493.

[5] *Berlin. klin. Wooh.*, July 13, 1903.

for such autumnal cases, and finds that golden-rod and ragweed contain toxines capable of exciting the disease. The toxine is not the same body as that found in grass-pollen, but the antitoxine prepared for the latter is capable of neutralising it to some extent. Dunbar considers that this action is comparable with the agglutinative action which the serum of a patient suffering from one disease may at times exert on bacteria other than the causal organism.

Dunbar adds some reasons for the want of success which at times attends the use of the serum as a cure for hay-fever. Patients often insist on sleeping with their windows open, and in otherwise exposing themselves to repeated infection with the toxine. They should, on the contrary, refrain from walks in the country at the times when they are liable to the disease, and generally avoid all opportunities of reinfection.

Dunbar's serum.—This is now prepared from horses, and is to be obtained commercially. It is called "Pollantin," and is sent out in small cases containing a bottle of the antitoxine and a drop-pipette for applying it. The following directions[1] for its use are supplied:—

"As the serum would soon be contaminated by frequent contact with the pipette, it is advisable to pour out one-third of the contents of the serum phial into the empty glass provided with a drop-pipette. This glass is effectually closed by pressing the indiarubber cap of the pipette into it, but care should be taken to keep this glass upright, so as to avoid the liquid flowing into the cap. Patients should not fail to carry a small quantity of serum in this glass about with them whenever they may expect a hay-fever attack. Immediately after noticing the first symptons of irritation in nose or eye, a drop or two of the serum should be instilled upon the eye or into the nose affected.

"The serum can be applied to the eye in the following manner:—After sucking up a few drops into the pipette,

[1] From Semon's article in the *Brit. Med. Journ.*, July 18, 1903, p. 124.

exert a gentle pressure on the rubber cap until one drop just emerges from the opening of the pipette; then before a mirror carefully approach the pipette to the outer corner of the eyelid, when the drop is sucked up by the eyelashes, and spreads over the conjunctival membrane. By the aid of a pocket mirror the same manipulation can easily be carried out in the open air.

"In order to instil the serum into the nasal cavity, fill the pipette with three or four drops of serum, and, bending your head backwards, insert the pipette about ½ in. into the nostril affected, and empty it by a short pressure on the rubber cap. A few sniffs suffice to spread the serum over the mucous membrane."

BOTULISM

The word "botulism," originally applied to any form of "sausage poisoning," is now confined to affections produced by eating meat contaminated with a particular micro-organism, the *Bacillus botulinus*, discovered by van Ermengem.

By growing the bacilli in broth and filtering the cultures, Kempner[1] obtained a toxine which, when injected into goats, produced an antitoxic serum. This was found capable of protecting rabbits against many times the lethal dose of the toxine, and also of exerting a curative effect if given some hours after the poison. No records of the use of this serum in human beings are available.

The toxine gives rise to changes in the cells of the spinal cord of a degenerative nature, similar to those found in fatal cases of the disease.

ANTIABRIN SERUM

Jequirity has been used in ophthalmic practice to induce inflammation in eyes which are affected with chronic indolent conditions (pannus, &c.). Difficulties frequently arise owing to the impossibility of graduating the amount

[1] *Zeitschr. f. Hygiene*, 1897, Bd. xxvi.

of inflammation produced. It has been found possible, however, by instillation of a serum[1] prepared by injecting animals with abrin, the active principle of jequirity, to control the inflammatory reaction produced by preparations of this plant.

GRAVES' DISEASE (EXOPHTHALMIC GOITRE)

In this disease the principle upon which serum-treatment is founded is different from that in most of those previously considered, since the serum is used to neutralise a poison formed by the cells of the patient himself, not by any parasitic organisms. The treatment is in reality of the nature of "organotherapy"—administration of animal organs or extracts. Since, however, it is serum which has been used in some cases, it demands notice here.

Ætiology.—The causation of this disease is not well understood. Serum-treatment is suggested on the ground that in all probability the disorder is due to an auto-intoxication caused by an excess of the secretion normally formed by the thyroid gland. The enlargement of this structure is a frequent, though not an invariable, feature of the disease, which may apparently exist in an abortive form, lacking any one or even two of its main features (tachycardia, exophthalmos, and enlargement of the thyroid gland).

Lanz's milk-treatment.—The first experiments in the direction of the treatment of Graves' disease by means of preparations derived from animals which had had the thyroid gland removed were made by Lanz,[2] who performed thyroidectomy on goats, and treated cases of exophthalmic goitre with their milk. He has recorded altogether six cases in which this treatment alone was adopted, and states that the results were very encouraging.[3] As an instance, we may quote the case of a woman, aged 38, who exhibited

[1] *See* Dieudonné, "Immunität, Schutzimpfung u. Serumtherapie," Leipzig, 1903, p. 121.

[2] *Correspondenzblatt f. Schweizer Aerzte*, 1899, No. 23.

[3] *Münch. med. Woch.*, 1903, p. 146.

the characters of the condition in a very marked degree. There were wasting and tremor, enlargement of the heart and tachycardia (160 per minute), and œdema of the lower limbs. Exophthalmos was well marked and v. Graefe's sign present. The characteristic thrill was perceptible over the thyroid gland, which was much enlarged. The patient was very weak and depressed, and slept badly. The milk-treatment was carried out in hospital for five weeks, at the end of which time very marked improvement had occurred. The woman felt much stronger, and could now walk about, which she could not do before. She was supplied with a goat from which the thyroid gland had been removed, and continued the treatment at home. At the end of a year she was apparently quite cured. Lanz states that he had never seen a cure effected previously in a patient who had reached the stage of the malady presented by this case.

Goebel[1] also has recorded a case of cure with the milk-treatment, but as the patient was given arsenic as well, it it impossible to be certain that the good result was due to the milk.

A desiccated milk derived from thyroidectomised animals has been prepared by Burghart and Blumenthal, under the name of "Rodagen." It is said to be efficient, but acquires an unpleasant cheesy smell and taste on keeping (Moebius).

Serum-treatment.—Ballet and Henriquez[2] and Burghart and Blumenthal[3] have used the serum of dogs from which the thyroid gland had been removed, for the treatment of the malady, and they all record good results. Moebius[4] has made use of the serum of rams, which had been similarly treated. Hypodermic injection was first

[1] *Münch. med. Woch.*, 1902, No. 20.

[2] *Semaine Méd.*, 1895, p. 329.

[3] *Deut. med. Woch.*, 1899, Nos. 37, 38; v. Leyden's "Festschrift," Berlin, 1902, p. 251.

[4] Schmidt's *Jahrbücher der ges. Medicin*, vol. cclxxiii., p. 43; *Münch. med. Woch.*, 1903, No. 4, p. 149.

employed, but was given up as unsatisfactory ; and administration by the mouth was substituted. Moebius gives 5 cc. of the serum every other day in a tablespoonful of wine. He noted a rapid diminution in the size of the thyroid gland in his patients, with diminished frequency of the pulse and cessation of the tremor. No ill effects were observed.

Schultes[1] also tried this remedy, giving smaller doses at shorter intervals (0·5 cc. three times a day). These amounts were gradually raised by daily additions of 0·5 cc. to each dose, till the patients were taking 4·5 cc. three times a day. Schultes first used sherry as a vehicle, and afterwards raspberry syrup. He noted the same good results as were claimed by Moebius, the pulse falling from 142 to 90 beats a minute, and the circumference of the neck diminishing to almost normal proportions.

Dreschfeld[2] records his experience of the serum in 21 cases, of which 10 were cured and 6 improved. He gave doses of 10 minims by the mouth thrice daily, gradually raising the dose to 20 to 25 minims.

Hallion[3] has used a glycerinated preparation of the whole blood of thyroidectomised animals (hæmatoéthyroidine), and a preparation known as "thyroidectin" is of somewhat similar constitution.

Stradiotti[4] prepared a *thyrotoxic serum* by injecting a sheep with powdered thyroid glands, and used it for the treatment of patients suffering from Graves' disease, the doses being from 5 to 45 cc. Subjective improvement and diminution of the tremor resulted, but the cardio-vascular condition was not influenced.

In America a serum prepared by Beebe is in use ; it is made by injecting an extract of finely powdered thyroid glands into animals, the extract containing nucleo-proteins

[1] *Münch. med. Woch.*, 1902, No. 20, p. 834.
[2] *Med. Chron.*, 1906, xliv. 203.
[3] *La Presse Méd.*, Nov. 1, 1905.
[4] *Riv. Crit. di Clin. Med.*, 1907, Nos. 7 and 8.

and globulins. The serum is standardised by measuring its power of agglutinating small fragments of thyroid gland.[1]

The exact mode in which the milk or serum acts is not quite clear. That it must somehow counteract the excess of thyroid secretion is evident ; but it can hardly be supposed that the mere injection of so small a quantity of serum free from this substance could reduce the proportion of it present in the blood of a patient suffering from Graves' disease to a point below that at which toxic symptoms occur. There must be some actively antagonistic principle in the serum or milk of the thyroidectomised animal which acts as antitoxine. It appears that symptoms of intoxication occur both when there is defect and when there is excess of thyroid secretion ; poisonous substances are set free which neutralise each other in health. In other words, the function of the thyroid gland must be the preparation of a substance which neutralises a poison formed by the other tissues of the body in the course of their activity. It would, however, be premature to form definite conclusions as to the ætiology of Graves' disease on the strength of the very small number of cases which are at present available as evidence of the curative properties of the serum of thyroidectomised animals. From the practical point of view, even should the treatment be proved successful, there would still remain the serious consideration of expense in connection with it. Moebius records that in one of his cases the cost worked out at 400 marks (£20).

Burkard[2] gave *diphtherial antitoxine* in doses of 3,000 units, and believed that it had a good effect. A similar observation was made by Legge.[3]

[1] *Journ. Amer. Med. Assoc.*, Sept. 1, 1906.
[2] *Ibid.*, 1906, Nov. 3.
[3] *Ibid.*, Apr. 22, 1905.

CHAPTER XV

ANTHRAX AND GLANDERS

ANTHRAX

Causal agent.—The *Bacillus anthracis* was the earliest micro-organism to be discovered, on account of its large size, the discovery being made by Davaine in 1850. The organism had probably been seen by Pollender in the previous year.

Of its *toxines* little is known; it does not even seem definitely settled whether it produces a soluble poison or only contains an intracellular toxine.

SERUM-TREATMENT

Sclavo's serum.—After a long course of experiments, Sclavo[1] succeeded in immunising goats against *B. anthracis*, and found that their serum had a protective and curative effect. The goats were injected first with attenuated cultures of the bacilli, and afterwards with virulent organisms. Rabbits were protected against lethal doses of anthrax-bacilli, and the injection of the serum within 24 hours after infection exerted a curative effect. Great difficulty is, however, experienced in preparing a serum sufficiently potent to protect against the more virulent strains of the organism. To test the value of the serum, rabbits are injected intravenously with 5 cc. of the serum, and then receive, two hours later, one-tenth part of a virulent agar-culture of *B. anthracis*.

More recently Sclavo has made use of asses for the preparation of his serum. It is found that if 2 cc. of

[1] *Berl. klin. Woch.*, 1901, Nos. 18 and 19, pp. 481, 520.

the serum thus obtained is injected into a rabbit along with 1 cc of a fresh, virulent broth-culture of bacilli, the animal is able to survive. For use in man, 30 to 40 cc. is injected in the flank, the whole amount being divided and injected in three or four different places. In severe cases, intravenous injection may be employed. The administration of the serum may be followed by a rise of temperature, which is of good prognostic import. Sclavo considers that his serum acts by stimulating the leucocytes in their conflict with the germs; in any case it is an antibacterial, not an antitoxic serum.

Very favourable results are recorded in cases of human anthrax in which Sclavo's serum was used. Thus Cigognani[1] records the successful use of this serum in a series of fourteen cases. Large doses were given without any ill effects beyond the appearance of urticaria. The amount used was 40 cc., which he found it best to give intravenously. Good effects are rapidly produced. The general condition improves in a few hours, and the pustules heal up within two days, while convalescence is much shortened. Cases which appeared alarmingly ill were cured by the use of the serum.

Legge[2] collected 67 cases treated with this serum; in 56 the serum was used alone. He quotes Sclavo's statistics of 143 cases, with a mortality of 6 per cent., as compared with the average mortality of 24 per cent. The serum should be given freely. It may be followed by a sharp rise of temperature (104°–105° F.). The serum is not bactericidal *in vitro*, and its exact mode of action is doubtful. Successful cases in this country are recorded by Lockwood and Andrewes,[3] Stretton,[4] and Mitchell.[5]

Mendez's serum.— Mendez[6] has immunised horses

[1] *Gaz. degli Ospedali*, 1902, No. 114.
[2] Milroy Lecture, *Brit. Med. Journ.*, 1905, p. 589.
[3] *Brit. Med. Journ.*, Jan. 7, 1905.
[4] *Lancet*, May 27, 1905.
[5] *Brit. Med. Journ.*, July 15, 1905.
[6] *Centralbl. f. Bakt.*, 1899, xxvi., Nos. 21 and 22.

by means of injections extending over periods of six to eight months, and finds that he obtains a useful serum. He records that in twenty-five cases in man the injections were followed by feeling of improvement, with fall of temperature, and subsidence of œdema and glandular swelling. The dose used is 20 cc., and it is seldom necessary to repeat it. In cattle and sheep the serum has a curative effect, acting efficiently even in doses of $\frac{1}{2}$ to 1 cc.

Deutsch's serum.—L. Deutsch[1] has also prepared a serum. It seems to be efficient for treatment of cattle, but has not been used upon man. It agglutinates the bacilli strongly, and contains an immune body or copula capable of destroying the organisms in the presence of a suitable complement.

Vaccination against anthrax has been carried out in cattle on a large scale with satisfactory results, but as it does not apply to human beings it cannot be dealt with here.

GLANDERS

Causal agent.—The *Bacillus mallei* was discovered by Loeffler and Schutz in 1882. Although primarily a disease of horses, man is highly susceptible—an average of four deaths per annum are recorded in England and Wales—and when attacked by the acute form almost invariably succumbs. Eyre[2] states that among 245 collected human cases, including both acute and chronic forms, there was a mortality of 85 per cent. In a series of chronic cases alone Bollinger states that 50 per cent. recovered.

Toxines.—By growing the bacilli for a month in flasks of veal broth at 37° C., then destroying the bacteria by heat and filtering the culture medium through a porcelain filter, a solution of toxines comparable to old tuberculin is obtained. This is known as mallein and is employed in the diagnosis of glanders in animals, but has no place in human medicine.

[1] Deutsch u. Feistmantel, *Impfstoffe u. Sera*, Leipzig, 1903.

[2] Art. "Glanders," Quain's "Dict. of Med.," 1902.

Serum.—No antiserum has been prepared for use in this disease.

Vaccine-treatment.—Wright[1] has treated one case of chronic glanders successfully with vaccine. We have treated one case with a vaccine prepared from the patient's own bacilli, with some improvement of the local lesions, but the "mallein" reaction at the site of injection of the vaccine and the rise of temperature were so marked that the patient soon refused to continue the treatment and discharged himself from the hospital.

Agglutination-reaction.—As *B. mallei* is agglutinated by the serum of infected animals and man, this reaction is valuable as a test for the presence of the disease. The microscopical method of observing agglutination, which is so reliable in the case of enteric fever, is however valueless here, as the reaction is tardy and the results inconsistent; but with the macroscopical reaction the results are quite trustworthy. For this purpose various dilutions of the suspected serum are made with normal saline solution by means of a graduated pipette; a homogeneous emulsion in sterile water of an agar-culture of *B. mallei*, 24 hours old, is also prepared, and equal quantities of the emulsion and of each of the dilutions are mixed either in small test-tubes or taken up in capillary pipettes and sealed. Control preparations with 1 : 5 and 1 : 10 solution of normal serum are likewise put up for comparison. The preparations are allowed to stand at room-temperature for 12 to 18 hours. At the end of the time they are examined, and those dilutions in which complete sedimentation of the bacilli has taken place are noted. Normal serum, even in 1 : 5 dilution, fails to produce sedimentation of the bacillus, whilst the serum of an individual infected with glanders gives a good reaction in 1 : 50 dilution, in many cases in 1 : 100, and sometimes in 1 : 200 and even higher dilutions.

[1] "Studies on Immunisation," 1909, p. 406.

CHAPTER XVI

MALIGNANT TUMOURS

Ætiology of malignant growths.—Nothing certain is known as to the causation of malignant tumours. The hypothesis that they are produced by the action of parasitic organisms appears to lack any satisfactory evidence and is insufficient to account for many of the peculiarities of new growths. Recently Sanfelice [1] has produced evidence in favour of the blastomycetic origin of malignant neoplasms, claiming to have produced both carcinoma and sarcoma experimentally by the injections of toxines of such micro-organisms, and to have cured these experimental tumours by the administration of an antitoxic serum prepared by immunising other animals by the injection of attenuated toxines.

But hitherto no generally accepted organism or toxine has been isolated with which an antitoxic or germicidal serum could be prepared. Treatment has, however, been tried on several different lines. On the supposition that a parasite is present in tumours, although it has not been discovered, some observers have tried injecting animals with portions of cancerous or other tumours, and applying the serum obtained from them to the cure of the disease. Reference has already been made in the introductory chapters of this work to the attempt to obtain, by injection of cancer-cells into animals, a cytolytic serum which shall be specific for these tumours; the practical results of this mode of treatment do not appear to have been very encouraging. The most hopeful line of treatment on bacterial lines is that inaugurated by

[1] British Medical Association Meeting, Belfast, 1909.

Coley, who employs the toxines of certain bacteria which seem to have a power of producing degeneration in the cells of tumours ; but it need hardly be pointed out that this mode of treatment is quite different from serum-treatment, or even from vaccines or toxines as applied to other diseases in which the specific organism is known.

COLEY'S FLUID

Principle of treatment.—It was observed many years ago that an attack of erysipelas occurring spontaneously in a patient suffering from malignant disease sometimes had the effect of causing a disappearance or retrogression of the growth. Attempts were therefore made to treat cases of cancer by artificial production of erysipelas, by inoculation with the streptococci. Some very successful results were obtained by Fehleisen both in sarcoma and carcinoma, and Coley and others also reported cases apparently cured by this means. Some fatal cases, however, occurred from this treatment, and it was abandoned as too dangerous. Coley[1] subsequently proposed to produce the same effect, but in a manner more under control, by means of injections of the toxines formed by the cocci in artificial media.

Preparation of the fluid. Coley at first obtained the toxines by growing together, in flasks of broth, cultures of the *Streptococcus erysipelatis* (*Streptococcus pyogenes*) and of the *Bacillus prodigiosus*, sterilising by heating to 58° C., and using the unfiltered fluid, containing the dead bodies of the organisms, for injection. For weakly patients and children Coley advised the use of the filtered culture, as being less toxic (in the proportion of 1 : 10).

Now, however, the fluid is prepared in a different manner.[2] The streptococcus is grown in broth for three weeks. To every 100 cc. of the culture is added 30 cc. of an emulsion of *B. prodigiosus* (standardised by Kjeldahl's method to contain 12·5 mg. of protein per cc.) and 20 cc.

[1] Art. "Erysipelas, Curative," in Quain's "Dict. of Medicine," 3rd ed., 1902, p. 486.

[2] *Proc. Roy. Soc. Med., Surgical Sect.*, 1909, July 13.

glycerin. A piece of thymol is added to the mixture, and the whole is sterilised by heating to 75° C. for two hours. Virulent strains of the streptococci are used for making the cultures, their vigour being maintained by passage through rabbits.

Dose of the fluid.—The initial dose advised by Coley is $\frac{1}{6}$ to $\frac{1}{4}$ minim, injected into the buttock with all antiseptic precautions; if injected into the substance of the tumour, the dose should not be more than $\frac{1}{16}$ minim. The injections may be repeated every two or three days. A temperature-reaction of about 102°–104° F. should be aimed at. So long as this is obtained, no increase in the dose should be made. When necessary, the increment should be $\frac{1}{4}$ minim. The biggest dose was usually 8 minims, though 20 minims have been given. If no good results are observed within the first three or four weeks, it may be concluded that the case is not suitable for the treatment. If, however, any diminution in the size of the growth is seen, the injections may be continued over long periods of time, until the tumour has entirely disappeared. Even then the injections should be continued in smaller doses at longer intervals for another four months. Occasional intermissions in the course of treatment are advisable.

Results of injections.—Coley[1] reported a total of 230 cases in which his treatment had been adopted, and among these two died as a result of the injections—one from accidental sepsis (staphylococcic), the other from embolism due to too large an initial dose. Thirteen patients had passed the three-year limit, generally considered to authorise the claim of "cure." More recently[2] he has reported a further series of cases, making the total up to 500, and including one more death from embolism. It also includes 52 inoperable cases which were successfully treated, and of these 35 remained well after from three to sixteen years. Recurrences took place in three cases which had shown definite improvement, thus attesting the correctness of the

[1] *Journ. Amer. Med. Assoc.*, 1900, i. 906. [2] *Op. supra cit.*

diagnosis. Coley also records 35 cases treated by other surgeons, with the result that in 26 the tumours disappeared, and 14 patients were alive from two to four years afterwards.

In other hands the treatment has not always proved so successful as would appear from Coley's results, but it may be pointed out that many of the failures date from fifteen years ago.

The cases best adapted for this toxine-treatment would seem to be the spindle-celled sarcomas, which are those least malignant in type and most nearly approaching organised tissue in their structure. Coley[1] collected 430 cases of sarcoma treated by this fluid: the tumours entirely disappeared in 47 (11 per cent.), and 28 of these cases were alive and well after from three to fifteen years. Melanotic sarcoma does not seem amenable to the injections. Coley does not recommend his treatment in cases in which operative removal of the growth is possible. In cases which are inoperable it appears well worthy of trial.

Coley explains the good effects seen after injection of toxines as being due to the induction of an environment unsuitable to the life of the cancer-cell, which degenerates in consequence. He holds that malignant tumours are produced by a parasitic organism which is affected by this treatment, just as cases of tuberculosis and of syphilis have been observed to show improvement after attacks of erysipelas. It does not appear necessary to see in Coley's results a support to the parasitic hypothesis. It is well known that the cells of tumours are of low vitality; and it is quite conceivable that they may succumb to the action of poisons circulating in the blood, when more resistant cells, such as those of normal tissues, are unaffected. It is also possible that the effect of the toxines is to supply in some way a stimulant to the normal connective tissue, and that its cells are enabled to offer a more vigorous resistance to the invasion of the tumour as the result of this stimulus. In tuberculosis and syphilis the action of

[1] *Boston Medical and Surgical Journal*, clviii., 1908, p. 175.

erysipelas must be exerted in the direction of an increase of tissue-reaction or possibly of phagocytosis, since it can hardly be maintained that the toxines of streptococci have a specific action on other organisms. The effect of bacterial toxines in inducing granular and fatty degeneration of tissues is well recognised, and the proneness of the cells of tumours to undergo these changes is noteworthy. That a conflict takes place between the healthy cells of the body and the invading cells of a tumour seems evident, not only on theoretical grounds, but on account of the signs of irritation and reaction seen at the periphery of a malignant growth. It is not impossible that both of the factors suggested may play a part in the action exerted by the toxines of erysipelas on tumours. It is noteworthy that the most marked effect is produced on the sarcomas which are connective-tissue tumours, and that the reaction which is called "inflammation," and which is induced by the action of bacterial toxines, is also seen in connective tissue.

SERUM-TREATMENT

Emmerich and Scholl's serum.—An attempt on rather different lines to utilise erysipelas as a cure for tumours was made by Emmerich and Scholl,[1] who inoculated sheep with the cocci of erysipelas, and used the serum of these animals for treatment of patients. Improvement seemed to result in some cases, but not actual cure. Reineboth [2] records a case in which this serum was used, and in which the growth showed signs of softening as a result of the injections; but the patient died in spite of the treatment.

Wlaeff's serum.—Wlaeff and D'Hotman de Villiers [3] obtained cultures of blastomycetes from cancerous growths, and with them inoculated pigeons. They then took the serum of these birds and tried it on rats as a protective against the form of cancer from which these rodents suffer.

[1] *Deut. med. Woch.* 1895, No. 17.
[2] *Ibid.*, 1895, No. 48.
[3] *Compt. Rend. de la Soc. de Biologie*, 1900, p. 611.

This serum has been used as a remedy for human cancer. Wlaeff reports that it causes the leucocytes to surround, penetrate, and destroy isolated epithelial cells. Reynier [1] reports that its use relieves pain and produces general improvement in patients, but that the growth of the tumours is not checked. Other cases with very similar results are recorded by Berger [2] and Richelot.[3] Lucas-Championnière [4] did not find that any benefit was derived from the injections in cases in which he tried it.

The idea that blastomycetes are the causal agents in cancer is not maintained by many authorities at the present day, and it is difficult to believe that any real effect can be produced in human cancer by such a serum as that just described. It is possible that a certain degree of irritation might be caused by foreign serum injected into a human being, and that some temporary effect might be induced in a tumour into which it was injected. Probably the only good results were owing to suggestion ; the patient was led to believe that some good would be done, and either imagined this or attributed some incidental improvement to the serum.

Doyen's antitoxic serum.—Doyen [5] has isolated from malignant growths an organism to which he gives the name of *Micrococcus neoformans*, and has prepared an antagonistic serum by injection of its toxines into animals. This serum he has employed for the treatment of cancer, injections being made into the buttocks. Of a total of 126 cases, 58 showed no improvement ; 18 cases were described as on the way to cure ; and 29 more had improved under the treatment ; in 21 cases the growth entirely disappeared. The serum was tried by Kirmisson [6] and by Morgan and Paine [7] with negative results.

[1] *La Semaine Méd.*, 1901, p. 59.
[2] *Ibid.*, 1901, p. 69.
[3] *Ibid.*, 1902, p. 142.
[4] *Ibid.*, 1900, p. 410.
[5] *La Presse Médicale*, 1904, No. 16 (*Tr. Soc. de Biol.*).
[6] *La Semaine Méd.*, 1905, p. 361.
[7] *Lancet*, April 7, 1906.

Schmidt's serum.—Schmidt,[1] of Cologne, prepared a serum by inoculating horses and sheep with cultures of a parasite derived from malignant growths. He claims good results from this preparation, but states that still greater benefit is derived from inoculation of the actual organisms themselves. The use of such a *vaccine* is followed by a reaction at the site of the growth, with some accompanying rise of temperature. The growth of the tumour is arrested, and retrogressive changes take place in the cells of which it consists. Schmidt maintains that the parasites isolated by other observers are all different forms of one organism, which is pleomorphic, and assumes different appearances according to its conditions of culture. Trial was made of Schmidt's serum in 9 cases at the Middlesex Hospital[2]; it was not found to influence the course of the disease.

Cytolytic serum.—Dubois,[3] who as early as 1897 injected macerated tumours into animals, and used the serum as a remedy, reported that fibrosis was thereby induced in new growths. Leyden and Blumenthal[4] endeavoured to prepare a cytolytic serum by injecting rabbits with finely-divided tumours taken from dogs. They considered that they had good results in dogs, affected with tumours, which received injections of the serum of the rabbits thus prepared. The serum was subsequently tried on human patients, carcinoma-cells from human sources being used for the preparation of the serum. The writers state that benefit was derived from the injections in some inoperable cases.

J.-B. Charcot[5] tried a similar serum, but admits that

[1] An account of Schmidt's work was given by Dr. H. J. Johnson at the Abernethian Society of St. Bartholomew's Hospital, Nov. 5, 1903. (See *Lancet*, Nov. 14, 1903, p. 1374.)

[2] *Lancet*, 1904, i., 684. *Cf.* Power (*Brit. Med. Journ.*, 1904, i. 299), who found that the serum produced an inflammatory reaction, but did not influence malignant growths.

[3] *Rev. Méd. de l'Est*, Feb. 1, 1897.

[4] *Deut. med. Woch.*, Sept. 4, 1902.

[5] *La Semaine Méd.*, 1900.

the results obtained were open to question. Some local reaction was produced by the serum at the seat of the tumour. The injections were not painful.

With regard to the use of serums of this nature, we are met by the difficulty that at present no proof is available that the cells of an epithelial tumour are of a different nature from those of the normal epithelium from which they are derived. Hence it is impossible to know whether it is practicable to produce effects on the cells of the tumour without the simultaneous occurrence of destructive changes in normal cells. The best hope in this respect seems to lie in the recognised lack of resistance met with in the cells of tumours, which, in spite of their rapid growth, or perhaps in consequence of it, are liable to undergo early degeneration. It is possible that a weak destructive force, such as might be supplied by an epitheliolytic serum, might suffice to kill tumour-cells, while unable to affect injuriously cells of normal resistance. The effects of the cytolytic serums at present prepared do not appear to be very potent.

CANCROÏN

Adamkiewicz [1] has prepared an extract of cancers with which he claims to have had astonishingly good results in cases of cancer. The extract is said to consist principally of neurine with some preservative fluid. He reported cases of cancer of the tongue, œsophagus, stomach, larynx, and breast, in which great improvement was effected by his preparation; and Kretzmer [2] records another case of œsophageal cancer which rapidly improved under injections of cancroïn.

Very severe criticisms of the cases recorded by Adamkiewicz were made by Nothnagel and others. It was pointed out that the diagnosis of cancer was not definitely made in any one of the cases. Carcinoma of the stomach is a condition which it is very difficult to diagnose with certainty;

[1] *Berlin. klin. Woch.*, 1902, No. 24.
[2] *Petersburg. med. Woch.*, 1902, No. 20.

and the patient who was said to be suffering from this disease and to be benefited by the cancroïn subsequently came back again for treatment for vomiting. In cases of cancer of the œsophagus, pieces of the growth may at times slough off and so leave a passage for food, rendering swallowing once more possible for a period of time. This may have occurred in the cases recorded above, and the temporary benefit have been ascribed to the cancroïn.

Poten[1] failed to obtain any improvement in two cases (cancer of breast and of uterus) in which he employed cancroïn. He points out that it is of no use to record cases of improvement under this or any other remedy, unless the diagnosis is confirmed by microscopical evidence.

It is almost impossible to believe that a remedy of the composition assigned to cancroïn can have any real effect on malignant growths. Much more definite evidence than that at present available will have to be forthcoming before the claims of this cure for cancer can be taken seriously.

VACCINE-TREATMENT

Doyen is perhaps alone in regarding the *Micrococcus neoformans* as the ultimate cause of malignant growths, but it is an undoubted fact that this variety of staphylococcus is very frequently associated with secondary suppurations, and the use of a vaccine in doses of 100 to 250 millions is under such conditions of considerable value. Jacobs and Geets[2] treated 37 cases of carcinoma of the breast with more or less improvement in 26.[3]

Summary.—The most remarkable fact about the various serums and remedies above alluded to is that they all seem to have produced good results in the hands of their inventors, but few, if any, of them appear to have succeeded in those of others. The only one which can be said to have established any pretence to efficacy is Coley's

[1] *Berlin. klin. Woch.*, 1902, No. 28.

[2] *Lancet*, 1906, i. 964.

[3] *See also* above, under "Schmidt's Serum," p. 347.

fluid, which has been tried now in a sufficient number of cases to afford material for forming a judgment. It appears to be definitely established that erysipelas may cause the disappearance of malignant growths, especially sarcomas; and the records of cases treated by Coley's toxines give ground for hope that the use of the toxines may be followed by similar good effect, without the dangers of the actual inoculation with bacteria. The treatment is certainly worthy of trial in cases which are beyond the aid of the surgeon. It is most important, however, to recognise that no time should be lost in medicinal treatment of any kind, if there is a possibility of removing the growth with the knife. This remains at present the only method which holds out reasonable hopes of cure in malignant disease.

CONCLUSIONS

1. Coley's fluid may be tried in inoperable cases of malignant disease, especially of sarcoma. It should not be used as a temporising measure in cases which are amenable to surgical interference.

2. Until it is proved that cancer is an infective disease, serum-treatment of the usual kind is inapplicable to this condition. It is, however, permissible to hope that it may be possible to produce a cytolytic serum which may act on the cells of the growth without affecting the normal cells of the tissues. A satisfactory serum of this nature does not appear to have been as yet prepared.

3. In secondary suppurative infections of malignant growths a vaccine of the responsible organisms—whether *Micrococcus neoformans*, or other bacteria—will frequently relieve certain of the more distressing symptoms.

APPENDIX I

THERAPEUTIC USE OF NORMAL SERUM (HORSE-SERUM), ETC.

Montgomery Paton,[1] as a result of trial of diphtherial antitoxic serum in diseases other than diphtheria, was led to believe that the serum or plasma of normal (untreated) animals had a definite effect in raising the power of resistance to infective conditions. He prefers, however, the serum of horses which have undergone a slight degree of immunisation against diphtherial toxines, as by this procedure, which acts by raising the resistance of the tissue-cells, the stimulating effect of the serum is increased. His work in cases due to pyogenic infection has already been mentioned (p. 228). He finds the use of normal plasma also valuable in tuberculosis, arthritis deformans, broncho-pneumonia, dysmenorrhœa, nephritis, cerebro-spinal meningitis, epilepsy, Graves' disease, traumatism, and other conditions. The serum is given by the mouth. The list of diseases for which it is useful is rather too long and varied for the claims made on its behalf to be very convincing.

Schmidt[2] and Borchardt[3] have injected normal serum into the peritoneal cavity before *operations* on the abdomen, with a view to increasing the resistance to any accidental septic infection; and Stuart Low[4] suggests the use of serum as a *dressing for wounds*. Hort[5] advises the use of serum, given by the mouth, in cases of *gastric ulceration*,

[1] "New Serum Therapy," London, 1906.
[2] *Deut. med. Woch.*, 1904, No. 49.
[3] *Ibid.*
[4] *Lancet*, 1907, i. 1221.
[5] *Ibid.*, 1908, ii. 462.

and H. D. Rolleston confirms his results so far as relief of pain and of hæmorrhage are concerned.

Latham[1] has used horse-serum by the mouth in cases of *pulmonary tuberculosis* along with tuberculin, and attributes good effects to the remedy. We have not been able to satisfy ourselves, however, that the serum has any definite value in this disease.

Weil[2] made trial of injections of the serum of normal individuals and of animals in cases of *hæmophilia*, with a view to supplying the coagulating substance which is wanting in these patients, and found that improvement resulted. His observations are confirmed by Broxa,[3] who states that normal serum may be given to these patients as a prophylactic against attacks of bleeding, and finds that diphtherial antitoxic serum, which is generally at hand, is effective.

It is worthy of note that Cole and Smirnow[4] find experimentally that foreign serum may in some instances favour infection. Thus if the serum of pigeons be injected into mice along with cultures of pneumococci, the animals succumb more readily to the parasite, though pigeons are themselves quite immune to the pneumococcus.

MORPHINE-ANTITOXINE, ETC.

It was stated in the first chapter of this book that the ordinary mineral and vegetable poisons, such as arsenic or morphine, do not give rise to the formation of antitoxines; and this is the view generally held. Hirschlaff,[5] however, claimed to have succeeded in producing an antitoxic serum which would counteract poisoning by morphine. He injected gradually-increasing doses of the alkaloid into rabbits, and obtained their serum. He then ascertained exactly the minimal lethal dose of morphine for a rabbit

[1] *Proc. R. Soc. Med.*, 1908.
[2] *Compt. Rend. Acad. des Sci.*, cxli., Nos. 15, 17.
[3] *Med. Klinik*, 1907, p. 1445.
[4] *Johns Hopkins Hosp. Bull.*, Sept., 1908, p. 249.
[5] *Berl. klin. Woch.*, Dec. 8 and 15, 1902.

per kilogram of body-weight, and found that if some of the serum was administered along with the poison, the animals were able to survive a larger amount of the poison than if no serum were given. The serum was also capable of protecting mice against the poison. Further, Hirschlaff found that if an emulsion of brain-substance was mixed with the morphine, larger doses of the latter could be tolerated, from which he concludes that the brain-cells have a power of fixing this poison similar to that which they exhibit towards the toxine of tetanus. In a (human) case of morphine-poisoning which he observed, Hirschlaff made use of some of the antitoxic rabbits' serum, and considered that it had a distinct antidotal action. He also found it useful in enabling patients who have formed a morphine-habit to leave off the drug at once, the severe nervous symptoms which usually occur in such circumstances not being experienced.

Hirschlaff's statements were severely criticised by Morgenroth,[1] and it is probable that his conclusions were founded on errors of observation.

An *anti-alcoholic serum* has been prepared by Sapelier and Dromard, but at present it is difficult to take the claims of the preparation very seriously.[2] The effects of serum derived from herbivorous animals in cases of *strychnine poisoning* have been investigated by Lo Monaco,[3] who suggests the use of diphtherial antitoxine in this condition.

[1] *Ibid.*, 1903, No. 21.

[2] An abstract of the authors' observations may be found in the *Journ. de Méd. et de Chir. Pratiques*, June 25, 1903, p. 461.

[3] *Arch. Ital. de Biologia*, June 10, 1903.

APPENDIX II

SINCE serums and other bacterial preparations are often needed for urgent cases, we have thought it well for purposes of ready reference to append a list of the various preparations with the names of the London firms supplying them. We have already pointed out that in the case of vaccines an autogenous culture should be preferred to a stock vaccine, but that the latter may be useful during the time of preparation of the former.

For the sake of brevity the different firms are denoted in the following paragraphs by initials only, thus:—

	Name of Firm.	*Telegraphic Address.*	*Telephone No.*
A. & H.	Allen & Hanbury's, Ltd. (Agents for the Lister Institute of Preventive Medicine)	Allenburys, London	2954 Avenue
A.-A. P. C.	Anglo-American Pharmaceutical Co. (Agents for the Lyons Pasteur Institute)	Anglo-Croydon	718 Croydon
B. W. & Co.	Burroughs Wellcome & Co.	Tabloid, London	13500 Central
C. R. A.	Clinical Research Association, Limited	Tubercle, London	8993 Gerrard
Mar.	W. Martindale	Martindale, Chemist, London	4688 Gerrard 1797 Pad'ngt'n
M. L. & B.	Meister Lucius & Brüning	Farbwerke, London	9992 London Wall
Mer.	E. Merck	Bissula, London	13736 Central
P. D. & Co.	Parke Davis & Co.	Cascara, London	8636 Gerrard 9201 Central
Reb.	Rebman, Limited (Agents for the Berne Serum Institute)	Squama, London	2026 Gerrard
Rob.	Roborat Co., Limited	Lecithin, London	9521 Central

ACNE.—Acne bacillus Vaccine and Mixed Acne and Staphylococcus Vaccine—**B. W. & Co., C. R. A., P. D. & Co.**

ANTHRAX.—Sclavo's Antiserum—**A. & H., Mer.**

BACILLUS COLI INFECTIONS. — Antiserum—**B. W. & Co.** Coli Vaccines—**B. W. & Co., C. R. A., Mar., Rob.**

CANCER. Coley's fluid **A. & H., P. D. & Co.** Neoformans Vaccine—**P. D. & Co.**

CHOLERA.—Kolle's Prophylactic Vaccine—**A. & H., Reb.**

CORYZA (Nasal Catarrh).—Catarrhalis Vaccine—**C. R. A., Mar., Rob.** Pneumobacillus Vaccine—**C. R. A., Mar., Rob.** "Combined" Vaccine—**Mar.**

DIPHTHERIA.—Antitoxic Serum—**A. & H., A.-A. P. C., B. W. & Co., M. L. & B., Mer., P. D. & Co., Reb.** Antidiphtheritic Pastilles—**A. & H., A.-A. P. C.**

DYSENTERY.—Antiserum—**A. & H., B. W. & Co., Reb.** Dysenteric Vaccine—**Mar.**

ENTERIC FEVER.—Antiserum—**B. W. & Co.** Wright's Prophylactic Vaccine—**A. & H., B. W. & Co., C. R. A., Mar., P. D. & Co., Rob.** Pfeiffer-Kolle's Prophylactic Vaccine, **Reb.**

GLANDERS.—Mallein—**A. & H., B. W. & Co., P. D. & Co.**

GONOCOCCUS INFECTIONS.—Antiserum—**B. W. & Co., P. D. & Co.** Gonococcic Vaccine—**A. & H., B. W. & Co., C. R. A., Mar., P. D. & Co., Rob.**

HAY-FEVER.—Dunbar's Serum (Pollantin) and powder. Willows, Francis, Butler & Thompson (171 London Wall).

INFLUENZA.—Vaccine—**C. R. A., Mar.**

MENINGITIS.—Antiserum—**B. W. & Co.**; (Ruppel's Serum) **M. L. & B.**; (Kolle's Serum) **Reb.** Meningococcic Vaccine—**Mar.**

PLAGUE.—Yersin's Antiserum—**A. & H., Reb.** Haffkine's Prophylactic Vaccine—**A. & H.** Kolle's Prophylactic Vaccine—**Reb.**

PNEUMOCOCCUS INFECTIONS.—Antiserum—**Mer., Reb.**; (Pane's Antiserum) **A. & H.**; (Romer's Antiserum) **A. & H.** Pneumococcus Vaccine—**A. & H., B. W. & Co., C. R. A., Mar., Rob.**

SNAKE-BITE.—Antiserum—**B. W. & Co.**; (Calmette's Antivenene) **A. & H.**

STAPHYLOCOCCUS INFECTIONS.—Antiserum, Polyvalent—**B. W. & Co.** Staphylococcus Vaccines—**A. & H., B. W. & Co., C. R. A., Mar., P. D. & Co., Rob.**

STREPTOCOCCUS INFECTIONS.—Antiserum—**A. & H., A.-A. P. C., B. W. & Co., M. L. & B., Mar., Mer., P. D. & Co.** Streptococcic Vaccines—**A. & H., B. W. & Co., C. R. A., Mar., P. D. & Co.**

TETANUS.—Antitoxic Serum—**A. & H., A-A. P. C., B. W. & Co., M. L. & B., P. D. & Co.**; (Tizzoni's Antiserum) **A. & H.**

THYROID.—Moebius' Antiserum—**A. & H., Mer.**

TUBERCULOUS INFECTIONS.—Marmorek's Antiserum—**Mar., Mer., P. D. & Co.** Tuberculins (concentrated)—**M. L. & B., Mer.**; (in dilution)—**A. & H., B. W. & Co., C. R. A., Mar., P. D. & Co., Rob.** Tuberculol—**Mer.** Ophthalmic test—**A. & H., B. W. & Co., M. L. & B.**; Calmette's—**Mar.** Cutaneous reaction—**A. & H., A.-A. P. C., Reb.**

NORMAL HORSE-SERUM—**A. & H., B. W. & Co.**

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Printed by Cassell & Company, Limited, La Belle Sauvage, London, E.C.

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